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THE VASCULAR SUPPLY OF THE BRONCHIAL TREE[1]

WILLIAM SNOW MILLER[2,3]

It is not my purpose at this time to discuss in its entirety the distribution of the bronchial artery within the human lung; its distribution to the bronchial tree, only, will be considered. In previous publications I have described other phases of its distribution.

The relation of the bronchial artery to the walls of the bronchi, and its ultimate distribution, have received scant attention from students of lung structure, and modern textbooks have but little to say on the subject.

It is, as has been said by other investigators, difficult to obtain complete injections of the bronchial blood vessels. This is due to two factors; first, the numerous connections between the bronchial blood vessels and the pulmonary veins; and, second, to the obstacle which the muscular coat, especially in the larger bronchi, presents to the free passage of the injection mass. This latter factor has been met with in the study of the blood vessels in the walls of other hollow structures; for example, by Mall, in his study of the vascular supply of the intestine.

The character of the injecting mass has also much to do with the successful isolating of the various systems of blood vessels found in the lung. If thin, fluid injections be used, it is not surprising that mistakes are made; for the ease with which they pass through the capillaries naturally leads to misconceptions. For this reason granular injecting masses which do not pass through the capillaries should be used; it was this type of injections that made the following study possible.

The bronchial arteries vary in number and origin. Usually three arteries are described; one for the right lung and two for the left lung. They ordinarily take their origin from the ventral side of the upper part of the thoracic aorta. The right bronchial artery is more variable in

[1] Read at the twentieth annual meeting of the National Tuberculosis Association, Atlanta, Georgia, 1924.

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[3] Aided by a grant from the National Tuberculosis Association.

its origin than are the left bronchial arteries; it may arise from the first intercostal artery, the third intercostal artery, the right internal mammary artery, or the right subclavian artery. Occasionally, all three arteries arise from a common trunk which has its origin on the ventral side of the thoracic aorta. Both sets of arteries pass along the posterior wall of their respective bronchus and enter the hilum of the lung.

As soon as the bronchial arteries have entered the lung they embed themselves in the layer of connective tissue which surrounds the bronchi. As a rule, two or three branches of the bronchial artery accompany each of the larger bronchi and bronchioli, and each subdivision of the bronchial tree is accompanied by a corresponding subdivision of the bronchial artery.

Anastomosing branches connect the arterial trunks with each other, and in this way a plexus is formed which has a much elongated and irregular mesh. The general course of the vessels which form this plexus is at a right angle to the muscular layer.

Branches of the bronchial artery can be followed as far as the smaller bronchioli. With the appearance of alveoli along the walls of the bronchioli, the character of the air-tubes changes; and the bronchial arteries, as a distinct set of vessels, disappear.

From the arterial plexus in the fibrous layer small branches are given off which penetrate the muscular layer; when they have reached the *tunica propria*, they turn and run for a short distance parallel with the muscle bundles, and then break up into capillaries which form a network, the long axis of which corresponds to the long axis of the bronchus.

From this network of capillaries venous radicles arise, which unite to form a plexus, with an irregular rectangular mesh, situated on the inner side of the muscular layer; from this plexus short branches penetrate the muscular layer, and form a second plexus of larger vessels along the boundary line between the muscular and fibrous layer. From this second plexus small veins arise which form one of the sources of origin of the pulmonary vein (fig. 5). By this arrangement the muscular layer of the bronchi and of the bronchioli is situated between two venous plexuses.

Taking up now in detail the distribution of the bronchial blood vessels, figure 1 is a tranverse section, 100 micra thick, of a bronchiolus 0.7 mm. in diameter. The three principal branches of the bronchial artery (red) are nearly equidistant from each other. Small branches of the artery appear scattered about in the outer fibrous coat of the bronchiolus. On the left side of the bronchiolus, midway between the pulmonary artery

(blue) and its lumen, some of the branches of the bronchial artery can be seen, which penetrate the muscular layer and eventually break up into the capillary network which is situated within the *tunica propria* of the bronchiolus. A few of these capillaries can be seen, as fine blue vessels, on the opposite side of the bronchiolus, running parallel to its long axis. The two venous plexuses, which enclose between them the muscular layer, also appear in this section, the outer plexus being made up of larger vessels than is the inner plexus. Small capillary vessels can be seen, here and there, extending between the venous plexus in the wall of the bronchiolus and the capillary network in the walls of alveoli which are in contact with the bronchiolus. The capillaries in the alveolar walls are derived from the pulmonary artery and not from the bronchial artery.

In figure 2, a portion of a bronchiolus, which has been cut open longitudinally, has been dissected in such a manner that the left leaf of the dissection is made up of the fibrous coat, while the right leaf is composed of the muscular layer, the *tunica propria* and the epithelium. The two leaves were then so placed that they could be opened like the leaves of a book. One of the leaves was turned to the left, while the other was turned to the right; by this procedure the left leaf lay with its outermost surface down, and the right leaf with its epithelial surface down.

The left leaf shows a portion of the arterial plexus, some of the smaller arterial branches, a portion of the outer venous plexus, and one of the venous radicles which would have eventually taken part in the formation of a pulmonary vein (see fig. 5).

The right leaf has a number of points of interest. A little to the left, and somewhat below its centre, a branch of the bronchial artery can be seen, situated underneath the point where three venous twigs apparently come together. The artery and the venous twigs were cut off from the arterial and venous stubs which can be recognized, nearly opposite to them, on the left leaf. The larger venules are part of the plexus outside the muscular layer; the smaller venules which, with the fine straight vessels, form the rectangular areas, belong to the venous plexus which is situated inside the muscular layer, while the fine straight vessels are the capillaries which are situated, just beneath the epithelium, in the *tunica propria*. It is necessary that the artery and its course in each leaf be recognized, for this is the artery that appears in figure 3, and gives origin to the capillary network there shown.

Figure 3 is an enlarged view of the lower half of the right leaf in figure 2. This was obtained by turning the leaf over, so that its epithelial

surface came to lie uppermost; for this reason the course of the artery appears to be reversed from that which it takes in figure 2. The dark blue vessels are the superficial capillaries; the lighter colored vessels are situated deeper, and take part in forming the venous plexus on the inner side of the muscular layer.

The artery is seen breaking up into a number of branches which at first run parallel to the muscle bands; they then give rise to a number of short branches, which terminate in capillaries that run lengthwise within the *tunica propria* of the bronchiolus.

I have already stated that with the appearance of alveoli along the walls of the bronchioli the bronchial arteries as a distinct set of vessels disappear. There is nothing new in this statement. That the *bronchioli respiratorii* and the air-spaces beyond them are supplied by the pulmonary artery and not by the bronchial artery, LeFort, Küttner, Schwarz and others have previously stated, and my own investigations confirm their observations.

As the *bronchioli respiratorii* are approached, the bronchial arteries terminate in a network of elongated capillaries which represent the capillaries in the *tunica propria* and the attenuated venules outside the muscular layer. By this arrangement the muscular layer is still enclosed by a vascular network. The capillary network thus formed quickly merges with the capillary network in the walls of the alveoli, and from this point onward the pulmonary artery alone supplies the air spaces.

Figure 4 shows a *bronchiolus respiratorius* which is dividing, on the right, into two *ductuli alveolares*. The section was of sufficient thickness to include the full diameter of the bronchiolus. In order to understand this section completely it is necessary to recall certain structural features of a *bronchiolus respiratorius:* the muscle is arranged in the form of anastomosing bands; opening out of the bronchiolus, through the mesh formed by these anastomosing bands, are the alveoli; the alveoli are supplied with blood by the pulmonary artery; and they take part in respiration.

The plane of the upper surface of the section in figure 4 is such that the muscle bands are intact, while the alveoli have been cut off close to their connection with the bronchiolus. That this is the case is shown by the clear openings leading into the bronchiolus. A branch of the pulmonary artery which was dividing into three smaller branches was cut through just as it arched over the rounded contour of the bronchiolus. If the capillaries, which are directly connected with that portion of the artery

which lies above the muscle in the centre of the section, be followed, it will be found that some of them pass beneath the bands of muscle while of others pass outside of the muscle; in this manner continuing the relation muscle and blood vessels which has existed throughout the bronchial tree.

The capillary network, which is in close apposition to any division of the air tubes, or to any blood vessel, is always coarser than in the walls of the air spaces. This is shown along the upper border of figure 4, where the capillaries are uniting to form a small venous radicle.

It is interesting to note that this transitional zone from the bronchial to the pulmonary circulation in the bronchial tree is a favorite place for tubercles to develop. I have found this true not only in experimental tuberculosis, but also in early human tuberculosis. Whether this be due to a retardation of the circulation at this point or to some other cause I am unable to state.

How is the blood which is brought to the bronchial tree by the bronchial artery carried away? In previous contributions on lung architecture I have shown that true bronchial veins are found only at the hilum of the lung, and that they arise from the first or the first two dividing points of the bronchial tree; they may also receive branches from that part of the pleura which is in close proximity to the hilum. These bronchial veins empty into the azygos, the hemiazygos, or one of the intercostal veins.

Within the lung the radicles which have their origin from the outer venous plexus in the walls of the bronchi and bronchioli, or from the capillary network in the walls of the *bronchioli respiratorii*, unite at the place where the bronchi or bronchioli divide to form a radicle of the pulmonary vein.

In figure 5 a branch of the pulmonary vein is shown, which has its origin from small venules that come from the place where two bronchi, which are respectively 3 and 4 mm. in diameter, arise from a bronchus which is 6.5 mm. in diameter. Another vein, which was formed in like manner, was present on the opposite side of the bronchus. As a rule, two radicles of a pulmonary vein arise from the place where bronchi or bronchioli divide. Smaller veins arise from the distal end of *ductuli alveolaris;* these are also paired, and they join larger branches of the pulmonary vein which are situated on the periphery of a primary lobule. To recapitulate: except for a limited area about the hilum, all the blood which is distributed to the bronchial tree is returned by the pulmonary veins.

Do anastomoses exist between the bronchial and pulmonary arteries? If the question be restricted to the arteries, I shall have to answer in the negative. If the question be asked if there be anastomoses between the bronchial and pulmonary circulatory systems, I shall have to answer in the affirmative.

Throughout the entire bronchial tree capillary anastomoses like those seen in figure 1 are found. It is conceivable that at times the same thing may occur in the lung as elsewhere in the body; namely, through some obstruction to the normal flow of blood a vessel, or vessels, may be developed to a greater degree than is the usual case, and in this manner an occasional anastomosis may arise.

Another source of misinterpretation is found in the fact that the *vasa vasorum* of the pulmonary artery are derived from bronchial arteries A branch of the bronchial artery is traced into the wall of a pulmonary artery; therefore they anastomose.

Up to the time of Guillot there was a general agreement that the bronchial artery formed an anastomosis with the pulmonary artery. Guillot found as the result of his studies that the two arterial systems were independent, and that injections made into the bronchial artery returned by the pulmonary vein. At first this radical change of opinion met with strong opposition, but later investigations have supported his statement.

When the richness of the capillary network in the bronchial walls is considered, it can readily be understood that it may be the source of a severe hemorrhage, arising either from a localized infection or from a foreign body finding lodgment in an air passage; it also assists in understanding how a localized infection in the wall of a bronchus or a bronchiolus may find its way through the epithelium, and gain entrance either into the vascular network in their walls, or into a lymphatic and thus give rise to an hematogenous or to a lymphogenous extension of the infection.

Wounds of the lung which involve the bronchial artery are much more fatal than those which involve the pulmonary artery; this is due to the higher pressure in the bronchial artery, which causes splenization or hemorrhagic infarction extending to the pleural surface, and is almost constantly provocative of empyema. Obstruction of the bronchial artery may occasion infarction and gangrene.

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DESCRIPTION OF FIGURES, PLATE 1

FIG. 1. Transverse section of a bronchiolus 0.7 mm. in diameter. Bronchial arteries, red; pulmonary vessels, blue. × 33.

FIG. 2. Dissection of a longitudinal strip of a bronchiolus. Bronchial vessels, red; pulmonary vessels blue. × 20.

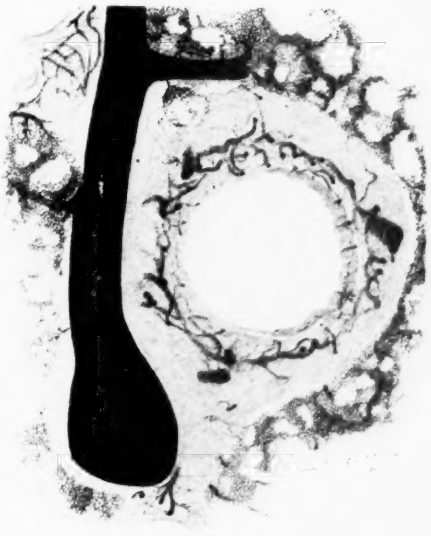


FIG. 1

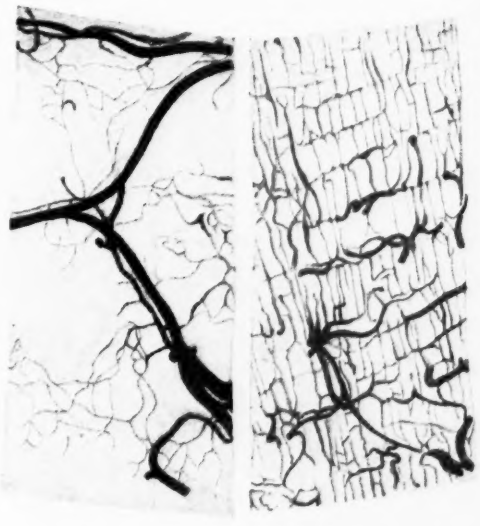


FIG. 2

DESCRIPTION OF FIGURE 3, PLATE 2

FIG. 3. Capillary network within the walls of a bronchiolus. Bronchial artery, red; capillaries, dark blue; venous plexus on the inner surface of the muscular layer, light blue. × 100.

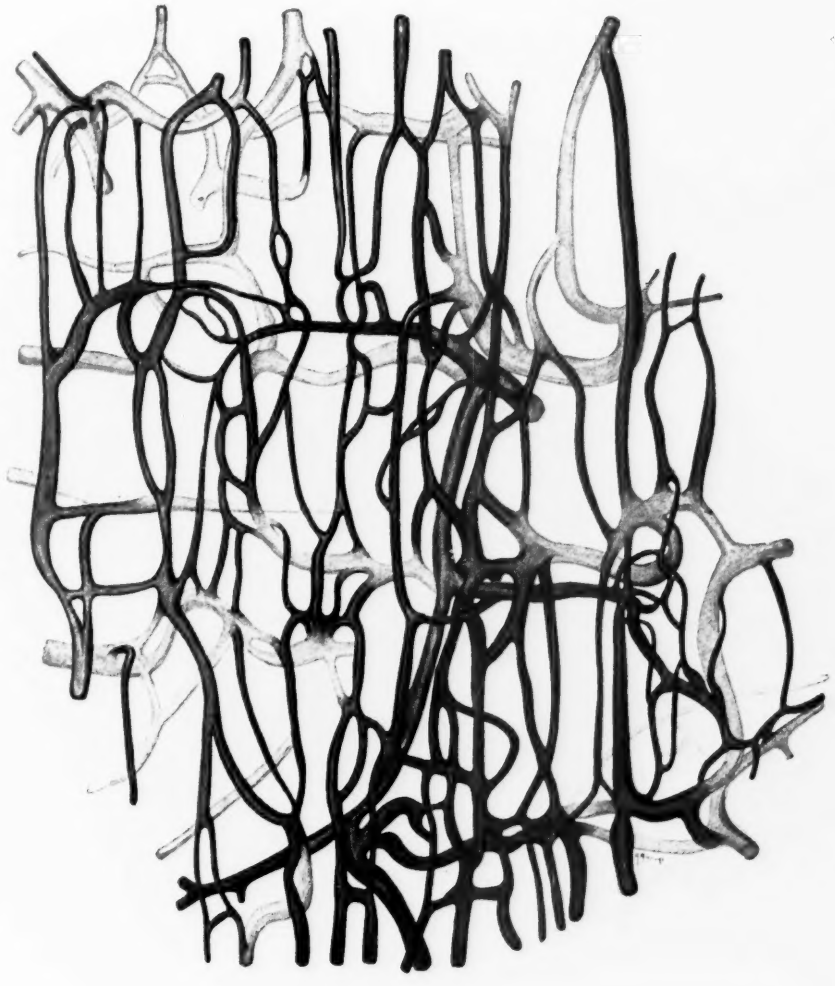


FIG. 3

DESCRIPTION OF FIGURES, PLATE 3

FIG. 4. Vascular supply of a bronchiolus respiratorius. Pulmonary artery, red; all other vessels, blue. × 65.

FIG. 5. A branch of the pulmonary vein arising from the place where branches are given off from a main bronchiolus. Bronchial artery, red; pulmonary vessels, blue. × 50.

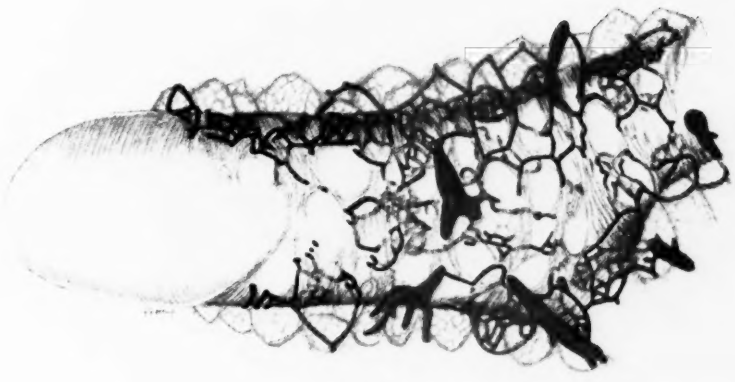


FIG. 4

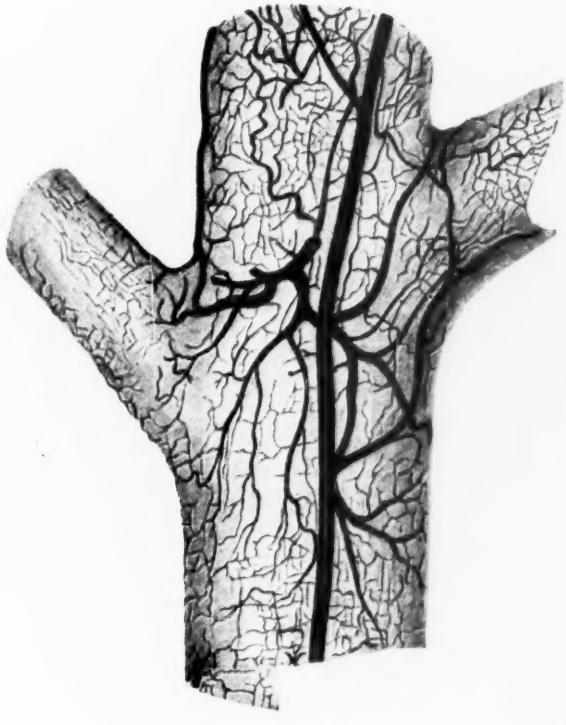


FIG. 5

ARTIFICIAL PNEUMOTHORAX WITH COLLAPSE UNDER LOW INTRAPLEURAL PRESSURES[1,2]

G. F. AYCOCK[3]

Since artificial pneumothorax became established in the treatment of pulmonary tuberculosis, certain disadvantages have been recognized as more or less necessary accompaniments. These are frequently of such importance as to justify serious consideration in determining the advisability of artificial pneumothorax in a given case, and, consequently, have limited the number of cases selected for this form of treatment according to the predominance of the operator's conservatism or enthusiasm.

It may be stated that complete collapse forms the basis for most of these objections, the most usual of which may be summarized briefly as follows:

1. *Displacement of the mediastinum and diaphragm:* The displaced mediastinum forces the heart to perform, under abnormal mechanical conditions, the extra work required of it, by reason of an increased resistance in the pulmonary circulation and by altered conditions of intrathoracic pressure, which in a measure interfere with the venous return to the right heart (1). There is also an interference with the compensatory hypertrophy and function of the contralateral lung. Diaphragmatic displacement, through its interference with the functional activities of the abdominal organs, gives rise to a chain of digestive symptoms, often very disagreeable to the patient, and is, no doubt, an important factor in the loss of weight usually experienced in the beginning of this form of treatment.

2. In many cases comparatively healthy lung is compressed along with diseased tissue.

3. An extra burden is thrown on the contralateral lung in performing the respiratory function, which may result in reactivation of a latent tuberculous process.

[1] From the Fitzsimons General Hospital, Denver, Colorado.
[2] Approved for publication by the Surgeon-General, U. S. Army.
[3] Major, Medical Corps, U. S. Army.

4. The relatively frequent development of accidental pneumothorax, which may result from one of the three following causes: (1) rupture of emphysematous blebs, (2) rupture of superficial cavities, and (3) rupture of pleural adhesions at their pulmonary attachments. The overzealous and unwise attempts to obtain the desired degree of collapse, through intrapleural pressures greater than conditions justify, are often responsible for the rupture of adhesions and superficial cavities. Accidental pneumothorax is followed by pyothorax in the majority of cases, which adds to the gravity of the situation.

5. The frequency of pleural exudates, the incidence of which is given at from 40 per cent to 100 per cent by the statistics of various authors.

6. The large refills after relatively long intervals may produce a disturbance in the intrathoracic equilibrium, which causes sudden changes in the relations of some of the important thoracic viscera, and the necessary readjustment frequently is accompanied by symptoms and signs of cardiac embarrassment.

7. At the termination of treatment the stretching, incident to reexpansion, is likely to cause a reactivation of the fibrosed lung. There is also considerable cardiac displacement, fixation of the lung by fibrous adhesions, and deformity of the chest wall on the treated side.

It would appear that most of these objections are built around faulty intrapleural pressures attained at refills, either directly or indirectly, and that any measures designed to overcome these unpleasant features of artificial pneumothorax must have as one of their main objects the proper regulation of intrapleural pressures.

This concept has been reflected in the recent literature, and particularly that of the last three years, in the pleas for partial collapse, lower intrapleural pressures, etc., which merit some discussion in passing.

Barlow and Kramer (2), in advocating a deliberate partial collapse which they term *Selective Collapse*, have described their method, which utilizes the apparent tendency of air, injected in small quantities and under low intrapleural pressures, to collect over the diseased portion of lung, producing collapse and allowing the healthy portion of the treated lung to function normally. This method, while having many advocates, has not escaped adverse criticism. The chief points of objection have been summed up by Riviere (3) as follows:

1. That it presupposes a free pleura, when the very nature of cases, which this method is designed to treat, is conducive to the formation of pleural adhesions which cannot be stretched by this procedure.

2. That the cases require so much individual attention as to limit the number one may treat.

Riviere further states that, in his experience with this form of collapse, it was the rule for the treatment to be jeopardized or cut short by the occurrence of "reactions" following the refills, and the development of a condition of "hypersensitiveness" to autotuberculin. This feature does not appear to have been noted by American authors.

Hennell and Stivelman (4) object to the term *Selective Collapse*, in directing attention to the fact that normal lung tissue, when collapsed by air, will show a reasonable degree of uniformity in compression, and, on absorption of the injected air, will show an equal uniformity in reëxpansion. This is explained by the uniform compressibility and elasticity of normal lung tissue. However, on attempting to collapse a partially diseased lung, with a free pleura, it is noted that the healthy portion of lung will show compression earlier than the diseased portion, but on absorption of the air the diseased portion will remain collapsed long after the healthy portion has reëxpanded. This is due to the loss of elastic recoil on the part of diseased lung tissue. This phenomenon may be observed in the behavior of the lung in the beginning and termination of any case of artificial pneumothorax.

We have endeavored to apply this pneumodynamic principle in our pneumothorax therapy at Fitzsimons General Hospital during the last three years. While our aim has been to accomplish the desired purpose by means of partial collapse in applicable cases, which fortunately has been the case with encouraging frequency, we realize that the nature of the distribution of lesions in many cases precludes the possibility of permanent satisfactory results on anything short of what is usually termed a complete collapse. What we have stressed is the importance of obtaining the desired degree of collapse, whether partial or complete, under the lowest possible intrapleural pressures capable of accomplishing a satisfactory result, the optimum pressure as described by the authors just referred to.

We measure a satisfactory collapse in terms of degree of cavity obliteration, that is, the approximation of cavity walls. Experience has taught us that unless large areas of cavitation are obliterated, or reduced to an insignificant degree of patency, no matter how striking the symptomatic results may be, the case must be carried under compression indefinitely, or the procedure abandoned in favor of one offering greater hope of a successful issue. If this satisfactory degree of collapse is not

obtained, cavities may be depended upon to enlarge to their former size on reëxpansion of the lung, in which event they are the menace they had previously been,—that of a potential chronic suppurative focus. So important a rôle does this factor of potential chronic suppuration play in the final outcome of a case, as witnessed in the degenerative visceral changes at necropsy, that, with a few exceptions, we have come to regard artificial pneumothorax solely in the light of its effect on cavities. There is a more immediate menace in partially compressed cavities, and this is the danger of accidental pneumothorax, especially if the cavities are superficial, not alone from the injudicious use of high intrapleural pressures, but from respiratory infections of a nontuberculous nature, which may invade the compressed lung, cause weakening of the cavity walls, and result in their rupture.

In contemplating a partial collapse, we recognize that, if a satisfactory compression of the diseased area is to be obtained, the collapse in the beginning must partake of the nature of a complete collapse, in that there is some lateral compression of the entire lung from apex to base. Hence, no thought of localization of collapse is taken until it is apparent that a satisfactory degree of compression of the diseased area will be obtained. When this degree of compression has been secured under frequent small refills at low intrapleural pressures, then the localization of collapse can be accomplished by allowing the healthy portion of lung to reëxpand, through lowered intrapleural pressures, and gradually increasing the intervals between refills. Thus, the desired degree of collapse is brought about through a "selective reëxpansion," if the term *selective* may be applied to the result of a natural sequence of events. By following this plan all the good qualities of a partial collapse may be obtained without carrying the patient through the succession of unpleasant features incident to mediastinal and diaphragmatic displacement, which are experienced under methods that bring about a more rapid and complete collapse.

As previously mentioned, it is necessary to give refills oftener and smaller than in the ordinary case of complete collapse. It is necessary to verify the degree of collapse and reëxpansion with frequent roentgenograms or fluoroscopic examinations. We believe the latter method to be more satisfactory in controlling reëxpansion, in that one can determine how near the expanding lung comes to the chest wall during inspiration. If this is not done, it is wise to take roentgenograms in both inspiration and expiration, to determine the degree of lung mobility.

We prefer not to allow the healthy portion of lung to reëxpand completely, on account of the probability of adhesions forming and closing up the air-space before we are ready for it.

It is quite natural that the value of such a procedure will be questioned in the presence of pleural adhesions, and it is true that adhesions, if present at all, will likely be located over the area desired to be compressed. However, Nature has dealt kindly in many of these cases, in that the adhesions are of sufficient delicacy to permit the pleural surfaces to be separated under low intrapleural pressures, with no apparent damage. It is very likely that the adhesions stretch under these circumstances and are not ruptured. In other cases, in which adhesions show greater density and a good lateral compression is prevented, injected air may find its way past the adhesions, anteriorly or posteriorly, compressing the apex from above downward, and at the same time giving a good anteroposterior collapse. Again, dense adhesions may be present laterally, with more delicate ones postero-mesially, which will stretch under low intrapleural pressures and thus give a good mesial compression.

Granting that none of these favorable conditions obtain, and the distribution of lesions is such that a partial collapse is indicated, the patient is none the worse off for our having attempted it. The question then arises as to whether his best interests will be conserved by attempting to bring about a successful collapse, through high, perhaps positive, intrapleural pressure. While we realize that authoritative precedent has been set for such action, and further that it is practised today by successful workers in this field, we must confess to the weakness of being influenced largely by our own experience, and feel a hesitancy in attempting it.

Considering the probability of rupturing adhesions at their pulmonary attachments, with attendant hemorrhage and effusion, or the rupture of superficial cavities with accidental pneumothorax, and its almost invariable accompaniment, pyothorax, to say nothing of other less disquieting features, we feel constrained to ask ourselves whether it is not better to abandon the hope of collapse, if it cannot be accomplished under moderate negative pressures after a fair trial, and to look to some other method of treatment for results,—for instance, extrapleural thoracoplasty.

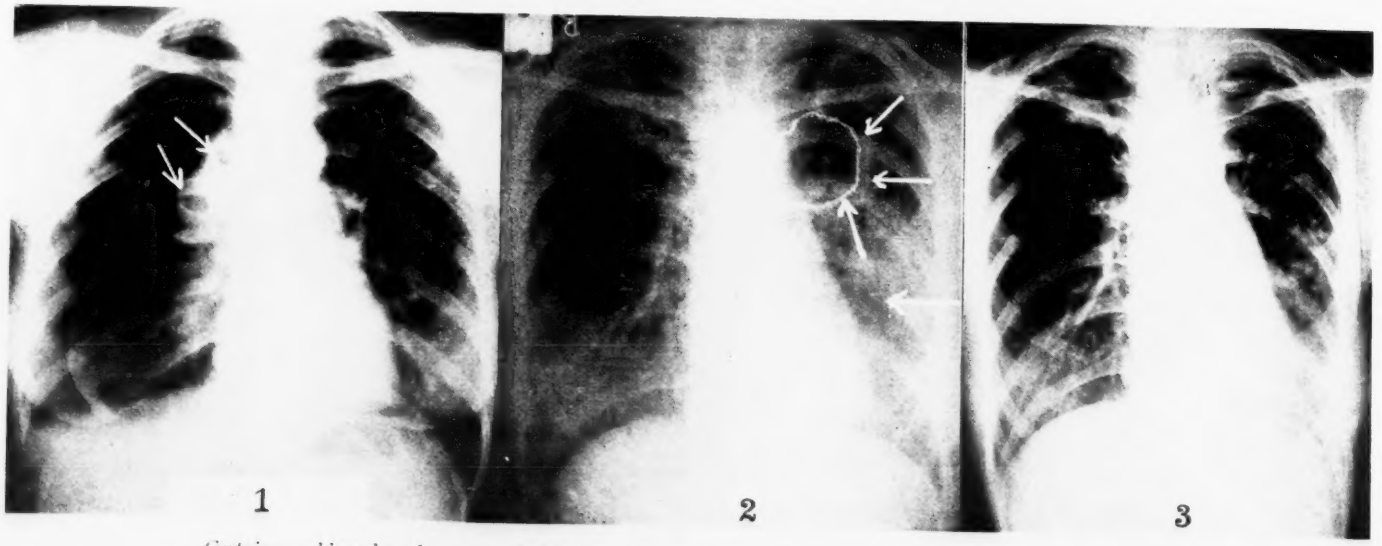
We believe these remarks can be applied with equal emphasis to cases for which, owing to a general distribution of lesions, a complete collapse is indicated. It is quite true that these cases, due to the nature and dis-

tribution of lesions, may resist compression for a considerable time, even when an apparently good pleural space has been obtained, and the tendency is to resort to positive pressures, especially if the presence of a fixed mediastinum is assumed. It is our belief that the majority of such cases, capable of showing a satisfactory collapse under any degree of compression, will do so under moderate negative-pressure inflations, frequently repeated. Undoubtedly, a longer time will be required to obtain a satisfactory collapse, but we believe this is more than compensated for by the absence of distressing symptoms and complications, which so often accompany a positive pressure collapse. The cases, which we have carried to complete collapse under this plan, have been virtually free from the tachycardia and dyspnea, or the gastrointestinal symptoms, that result from mediastinal and diaphragmatic displacement incident to rapid collapse under positive pressures.

Another good feature we have noted, in cases of both partial and complete collapse, and this is the lowered incidence of pleural exudates under low intrapleural pressures. This agrees with the experience of others (2) (4). Especially is this true as regards exudates of large quantity. Just to what extent pleural exudates are due to rupture of pleural adhesions, as assumed by Barlow and Kramer, we are not prepared to state, but we can recall cases in which effusions developed immediately after the rupture of adhesions, as shown by roentgenograms, with such regularity as to place them beyond the bounds of coincidence.

Brief reference will be made to four cases, with photographic reproductions of roentgenograms, to illustrate certain points brought out in in this discussion.

Case 1. (Represented by fig. 1.) This case is presented for the purpose of showing how patent cavities in the compressed lung may be an immediate menace. Compression was done in this case on account of a very recent nodose involvement, with marked perifocal reaction, in the upper half of the right lung. Satisfactory partial collapse was obtained, except that the cavities were not completely collapsed. The patient showed a decided symptomatic improvement, and progressed favorably until she developed a cold, which was followed by an acute infection of the compressed lung, of probable pneumococcus origin, which no doubt weakened the cavity walls, causing rupture. The accidental pneumothorax resulting was soon followed by an empyema, from which the pneumococcus was isolated in pure culture.



Certain markings have been retouched in the photographs for the purpose of bringing out radiographic detail

FIG. 1. Case 1. Superficial cavities, not completely collapsed, ruptured after acute infection of compressed lung with accidental pneumothorax, followed by empyema from which the pneumococcus was isolated in pure culture

FIG. 2. Case 2. Before compression. Note cavity about 5 cm. in diameter in postero-mesial aspect of left upper lobe and conglomerate nodose lesions with marked perifocal reaction below.

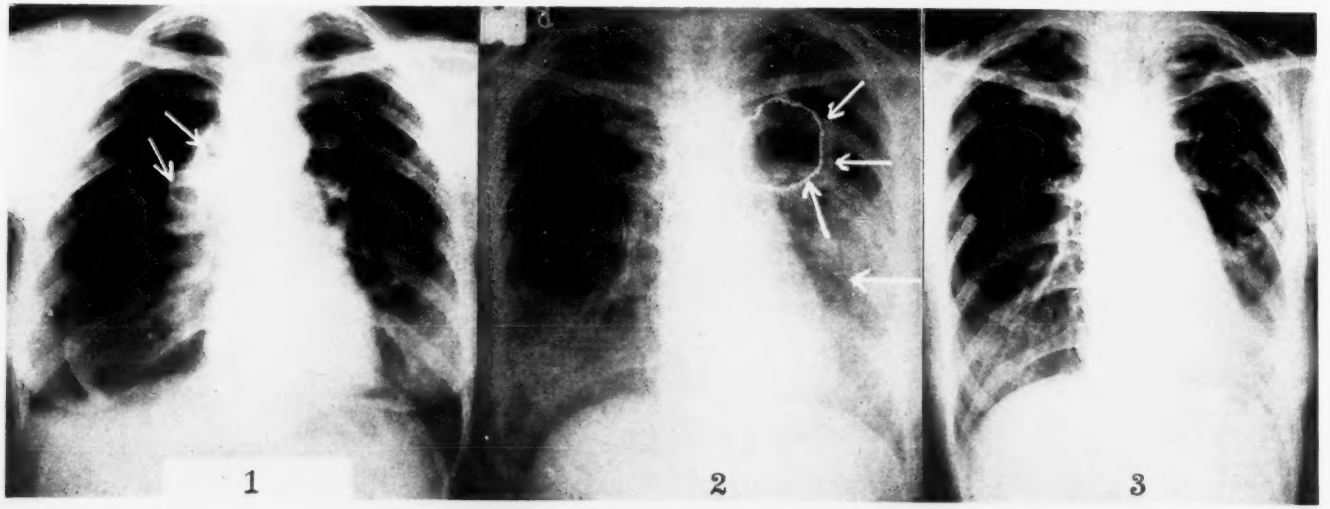
FIG. 3. Case 2. Same as fig. 2 after complete reëxpansion. Note absence of large cavity and clearing up of perifocal reaction, even after making allowances for difference in exposure of the two films.

tribution of lesions, may resist compression for a considerable time, even when an apparently good pleural space has been obtained, and the tendency is to resort to positive pressures, especially if the presence of a fixed mediastinum is assumed. It is our belief that the majority of such cases, capable of showing a satisfactory collapse under any degree of compression, will do so under moderate negative-pressure inflations, frequently repeated. Undoubtedly, a longer time will be required to obtain a satisfactory collapse, but we believe this is more than compensated for by the absence of distressing symptoms and complications, which so often accompany a positive pressure collapse. The cases, which we have carried to complete collapse under this plan, have been virtually free from the tachycardia and dyspnea, or the gastrointestinal symptoms, that result from mediastinal and diaphragmatic displacement incident to rapid collapse under positive pressures.

Another good feature we have noted, in cases of both partial and complete collapse, and this is the lowered incidence of pleural exudates under low intrapleural pressures. This agrees with the experience of others (2) (4). Especially is this true as regards exudates of large quantity. Just to what extent pleural exudates are due to rupture of pleural adhesions, as assumed by Barlow and Kramer, we are not prepared to state, but we can recall cases in which effusions developed immediately after the rupture of adhesions, as shown by roentgenograms, with such regularity as to place them beyond the bounds of coincidence.

Brief reference will be made to four cases, with photographic reproductions of roentgenograms, to illustrate certain points brought out in in this discussion.

Case 1. (Represented by fig. 1.) This case is presented for the purpose of showing how patent cavities in the compressed lung may be an immediate menace. Compression was done in this case on account of a very recent nodose involvement, with marked perifocal reaction, in the upper half of the right lung. Satisfactory partial collapse was obtained, except that the cavities were not completely collapsed. The patient showed a decided symptomatic improvement, and progressed favorably until she developed a cold, which was followed by an acute infection of the compressed lung, of probable pneumococcus origin, which no doubt weakened the cavity walls, causing rupture. The accidental pneumothorax resulting was soon followed by an empyema, from which the pneumococcus was isolated in pure culture.



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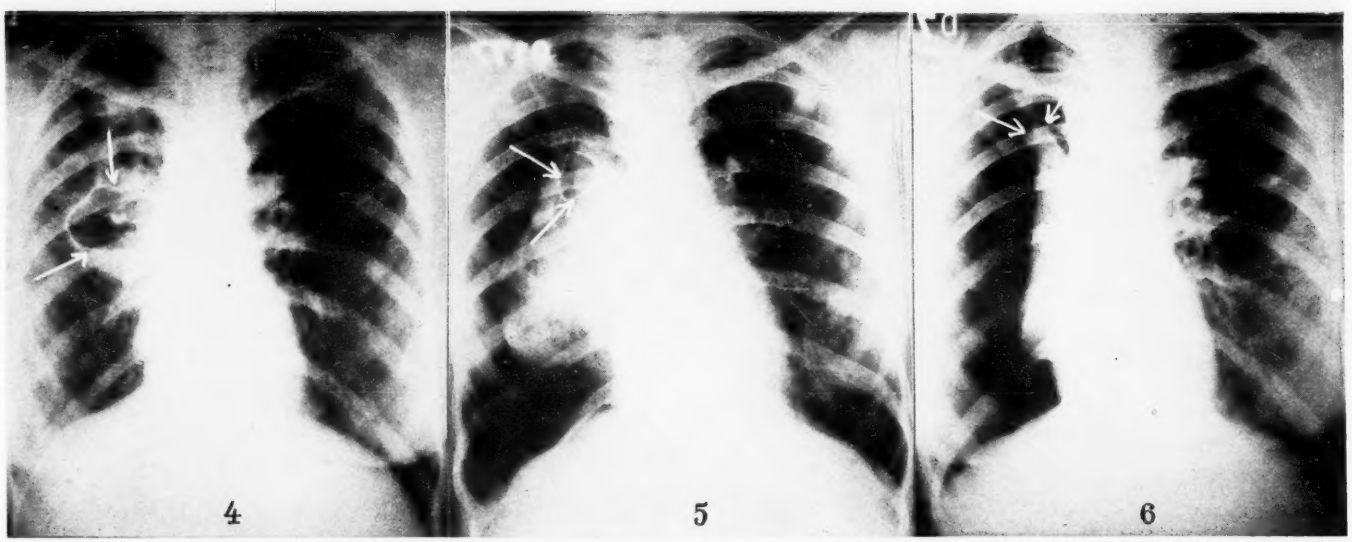

FIG. 4. Case 3. Prior to compression. Extensive lesion, largely fibrous in type, with cavity 5 cm. in diameter in mesial aspect of midlung.

FIG. 5. Case 3. Three months after beginning of artificial pneumothorax. The cavity is still patent to a considerable degree to its own rigid walls and adhesion opposite first interspace anteriorly.

FIG. 6. Case 3. About one year after beginning artificial pneumothorax. Notice complete collapse of cavity through lateral and mesial compression.

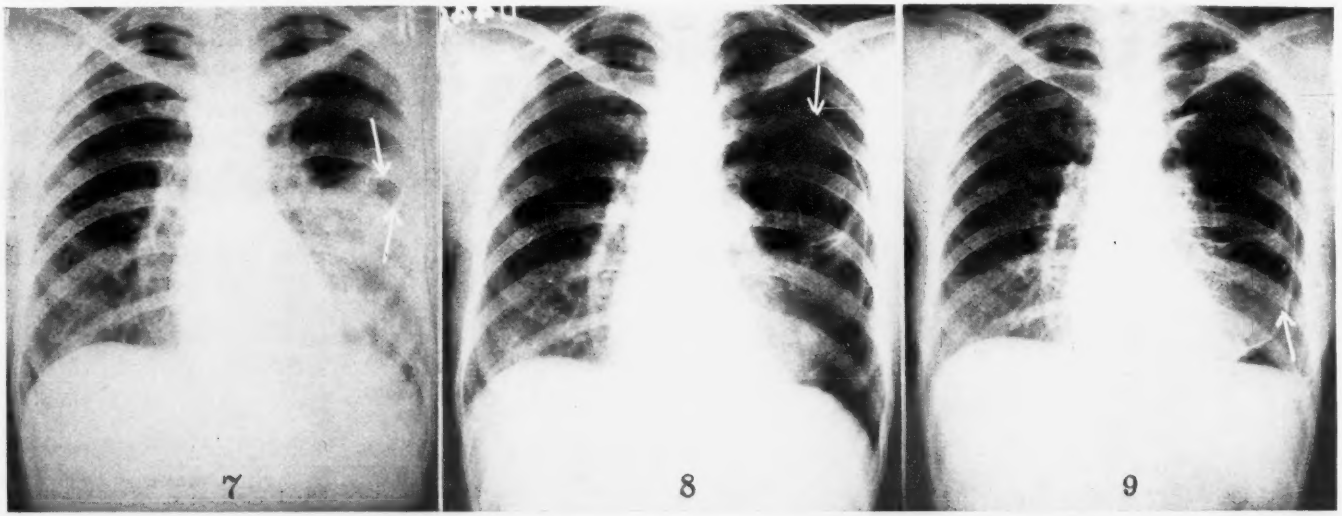

FIG. 7. Case 4. Before compression. Recent nodose involvement of left lower lobe with marked perifocal reaction: cavity 3 cm. in diameter in upper portion of involved area.

FIG. 8. Case 4. One month after beginning artificial pneumothorax. Cavity almost completely obliterated.

FIG. 9. Case 4. Nine months after beginning of pneumothorax. Slight upper lobe collapse in this case was intentionally maintained on account of adhesions over base of lung, interfering with refills. Note absence of cavity and clearing up of tuberculous process, apparently by resolution.

Case 2. This case showed a cavity about 5 cm. in diameter, in the postero-mesial aspect of the left upper lobe, below which were conglomerate nodose lesions with marked perifocal reaction, extending well down into the upper portion of the lower lobe. An apparently healed lesion was present in the upper portion of the right upper lobe. It was readily apparent that a complete collapse was indicated in this case. Compression was begun in April, 1922. Moderate negative-pressure refills were given over a period of three months, at intervals of four to eight days, before a satisfactory collapse was obtained. There was no appreciable mediastinal and diaphragmatic displacement, and the patient's clinical course has been uneventful since satisfactory collapse was established. Figure 2 represents the chest condition prior to collapse, and figure 3 the condition after reëxpansion, about January 1, 1925. It will be noted that there is no evidence of the presence of the large cavity existing prior to compression, the perifocal reaction around the lower lobe lesions has cleared up, the position of the mediastinum and diaphragm will compare favorably with that existing prior to compression, and there is no more chest deformity than one would expect from any fibrous lesion of this extent.

Case 3. This patient had an extensive right-lung lesion, with a large cavity about 5 cm. in diameter, in the mesial portion of the midlung. The left lung showed no definite evidence of a clinical tuberculosis. Compression of the right lung was begun in September, 1923, but it was soon apparent that the cavity could not be collapsed readily, due to its own thick walls, and a very dense adhesion opposite the first interspace anteriorly. Figure 4 represents the X-ray findings prior to compression, and figure 5 the state of collapse after the lung had been under compression for three months. It will be noted that the cavity is still patent to a considerable degree. Figure 6 represents the state of collapse about one year after the beginning of treatment. There is both lateral and mesial compression, as well as some compression from the apex downward. This degree of collapse was obtained through negative-pressure refills at six to eleven-day intervals.

Case 4. This case presents three points of unusual interest. First, that it is a case of lower lobe involvement (left), the remainder of the lung being apparently healthy; second, that it is perhaps the result of the breaking down of a latent focus which has become reactivated, probably a tracheobronchial lymph node (this position is strengthened by the appearance of the small annular shadow in figure 9 which may be assumed to be the remnants of the node after the caseous products had been thrown off); third, that it illustrates the value of partial collapse.

As will be noted from figure 7, this patient had a recent nodose lesion, with considerable perifocal reaction, in the greater portion of the left lower lobe,

and a cavity about 3 cm. in diameter in the upper portion of the involved area. Figure 8 shows the successful partial collapse, obtained within thirty days after treatment was begun. It is apparent that there is no disturbance in the position of the mediastinum and diaphragm, and the cavity has been collapsed completely from an X-ray standpoint. Figure 9 will show the appearance of the collapsed lung nine months after the beginning of treatment. In spite of the fact that the diseased area is in a state of partial collapse, it is readily discernible that there has been an almost complete clearing up of all X-ray indications of tuberculosis, apparently by resolution. The possibility of determining the progress in the compressed area is an advantage of partial collapse not often mentioned.

SUMMARY

1. We feel that most of the unpleasant features of artificial pneumothorax result, directly or indirectly, from the employment of faulty intrapleural pressures,—usually positive pressures.

2. Partial collapse is the method of choice in cases suitable from a viewpoint of extent and nature of involvement, as in this form of collapse two ideal conditions are provided, namely (1) the diseased area of lung is placed at rest, and (2) the maximum of healthy lung tissue is permitted to function.

3. Cases, capable of being compressed at all, will with few exceptions show satisfactory collapse under moderate negative intrapleural pressures, with frequent small refills. If satisfactory collapse is not obtained under this plan, the majority of cases will not be more successful after attempts under positive pressures, and, considering risks incurred, will doubtless fare better under other procedures.

4. Artificial pneumothorax may be considered successful only in so far as it accomplishes obliteration of cavities. Cavities remaining patent to any extent are likely to attain their former size on reëxpansion of the lung, the lesion becoming reactivated, with a *status quo ante;* namely, the presence of a potential chronic suppurative focus, which leads to the degenerative visceral changes, causing the majority of fatalities in tuberculosis.

5. Repeated roentgenograms and fluoroscopic examinations are absolutely necessary if artificial pneumothorax is to be successful, particularly in the types of collapse in which frequent small refills under low intrapleural pressures are to be used.

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THE CIRCULATORY CHANGES IN ARTIFICIAL PNEUMOTHORAX[1]

RAPHAEL A. BENDOVE

When the lungs are subjected to conditions in which their respiratory area becomes diminished and oxygenation deficient, circulatory changes are likely to ensue. In pneumothorax a great part of the respiratory capacity is thrown out of function, and this investigation of the circulatory changes following its institution in certain cases of pulmonary tuberculosis was pursued with the aim of finding out whether these alterations have any bearing on the course and result of the treatment.

The report includes 14 cases, the circulatory and blood changes of which were studied before and after the induction of artificial pneumothorax and their blood pressure taken for months before and after each inflation; 2 cases of spontaneous pneumothorax; and 9 cases the blood pressure of which began to be observed only after the pneumothorax had been established for months or years but of which heart and lung changes could be studied from the roentgenograms taken prior to and after the initiation of the treatment.

The following changes were observed:

Blood pressure: An increase in the blood pressure after the induction of pneumothorax was found in 8 cases, the increase ranging from 15 to 40 mm. Hg. in systole and 10 to 25 mm. Hg. in diastole, and it lasted about an hour after the inflation; 4 cases showed no change at all, and 2 cases had a slight fall in the blood pressure after the introduction of gas into the pleural cavity. No appreciable change in the blood pressure after the inflations was observed in those cases in which the pneumothorax had been established for more than two months.

Increase in the diameter of the heart as studied röentgenographically: The 8 cases which had an increase in the blood pressure after the initiation of pneumothorax developed an enlargement of the heart about ten days after the beginning of the treatment. One case with a fall in blood pressure also showed an increase in the transverse diameter of the heart.

[1] From the Montefiore Sanatorium for Tuberculosis, Bedford Hills, New York.

The other 5 cases exhibited no material change in the size of the heart. Of the 9 old cases studied there were not many X-Ray plates taken immediately after the induction of the pneumothorax, but of the few plates found 4 showed a decided cardiac enlargement.

Of particular interest was an observation made in 4 cases in which the pneumothorax therapy was about to be completed and the treated lung was expanding and functioning almost to its physiological limit. In these the heart, previously enlarged, became reduced in size, though it remained a little larger than it was prior to the initiation of the treatment. (Compare figs. 1, 2 and 3.)

Pulmonic second sound: Accentuation of the pulmonic second sound was found in those cases which developed cardiac enlargement and a rise in blood pressure after the institution of pneumothorax. In the case which showed a definite increase in the transverse diameter of the heart but no rise in blood pressure after inflation, the pulmonic sound was not accentuated.

Compensatory emphysema: Marked compensatory emphysema was noticed in all those cases which exhibited a definite increase in the size of the heart and had a rise in blood pressure after the induction of pneumothorax. This vicarious emphysema was most pronounced in cases in which the treated lung was almost totally compressed because of high intrapleural tension or development of fluid (see figs. 4 and 5). Those cases treated with expansile pneumothorax, that is, in which the intra-

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FIG. 1. DENSE INFILTRATION AND FIBROSIS OF RIGHT UPPER THIRD OF LUNG. COMPENSATORY EMPHYSEMA OF LEFT LUNG

FIG. 2. PLATE OF SAME PATIENT AS OF FIGURE 1, TAKEN FIVE WEEKS AFTER INDUCTION OF PNEUMOTHORAX. RIGHT PNEUMOTHORAX AFFECTING A MUCH GREATER COMPRESSION OF THE UPPER DISEASED PORTION OF THE LUNG

Note particularly the marked increase in the size of the heart

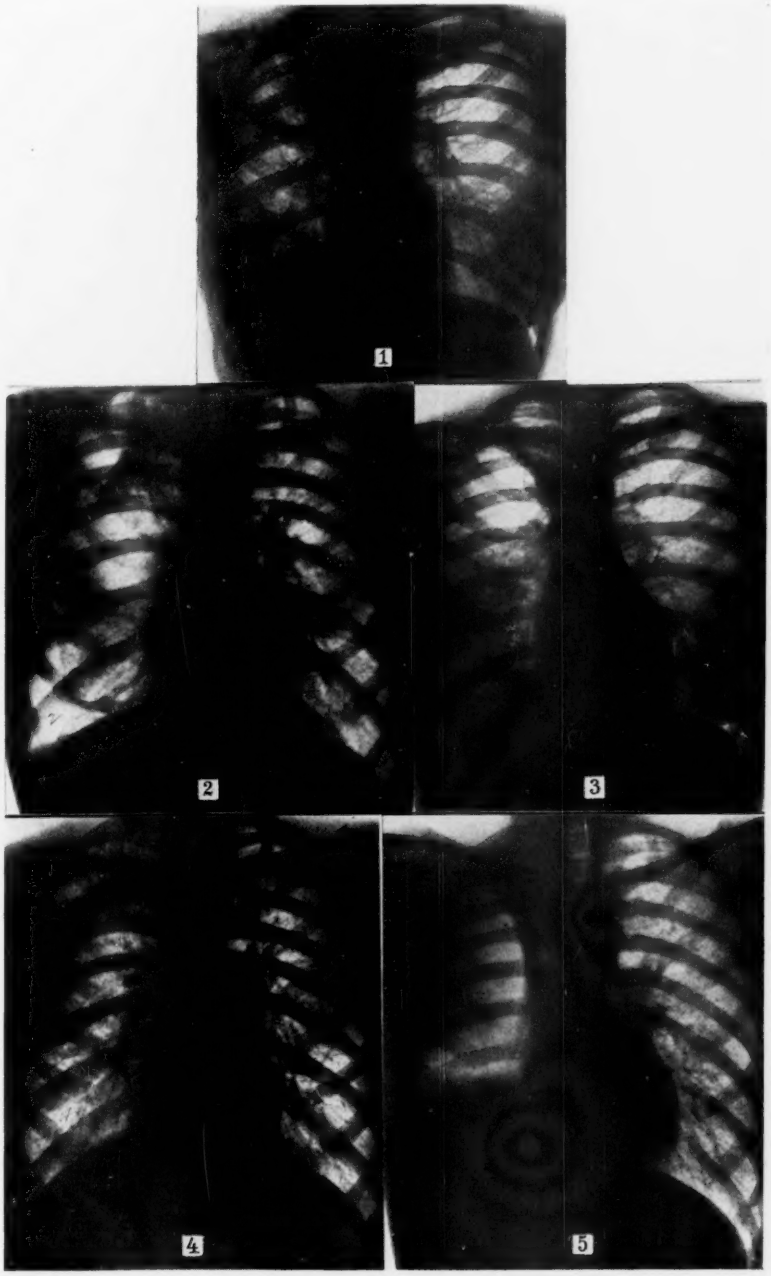
FIG. 3. PLATE OF SAME PATIENT AS OF FIGURES 1 AND 2, TAKEN AT TERMINATION OF TREATMENT

Note the compensatory emphysema in both lungs. The size of the heart is smaller than in figure 2, but it still appears larger than in figure 1.

FIG. 4. SCATTERED INFILTRATION OF RIGHT LUNG; HEART PROMINENT

FIG. 5. PLATE OF SAME PATIENT AS OF FIGURE 4, TAKEN ABOUT FOUR MONTHS LATER. HYDROPNEUMOTHORAX COMPRESSES THE ENTIRE RIGHT LUNG

Note increase in size of heart, and particularly the marked compensatory emphysema developed in the left lung, its longitudinal diameter being about two inches longer than in the previous plate.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pleural pressures were always negative, and the uninvolved portion of the treated lung expanded and contracted with each respiratory phase, developed compensatory emphysema also in this functioning portion of the lung. The vital capacity was higher in these cases than in those which did not develop compensatory emphysema.

Blood changes: Blood counts and hemoglobinometry were done before and after the inflations, but the work was not carried out systematically and we could arrive at no definite conclusions, though as a whole there seemed always to be an increase in the number of red blood cells and the hemoglobin percentage after the induction of pneumothorax.

The relation of these changes to the result of the pneumothorax treatment could be summarized as follows: (1) Those cases, which exhibited a definite cardiac enlargement and a rise in blood pressure after the first few inflations, developed also a marked compensatory emphysema and as a rule did well; the pulmonic second sound was invariably accentuated and the vital-capacity reduction was never equal to the same amount of air introduced into the pleural cavity. These were young adults who gave a history of short duration of illness, but had a stormy clinical course, and whose lesion was of a moderate or moderately advanced degree and not of the fibroid type. Their blood pressure before the induction of pneumothorax was within physiological limits. (2) Those cases, which showed no increase in size of heart and no elevation in blood pressure after the first inflation, did not do very well; the pulmonic second sound was never accentuated and compensatory emphysema was only slight or absent altogether. They were middle-aged people who had been sick for many years, and had advanced lesions of the fibrocaseous type. Their blood pressure before the initiation of pneumothorax was low and the vital capacity very small. The case which showed cardiac enlargement but a fall in blood pressure after the inflations, developed an effusion very soon which later turned into pyopneumothorax.

Of the 2 spontaneous pneumothorax cases, one died a few days after its occurrence; the respiration was labored, the pulse very rapid and the blood pressure 90/40, there was no accentuation of pulmonic second sound, and displacement but no enlargement of heart was noticed. The other patient did well, and the pneumothorax was kept up artificially; he developed a marked vicarious emphysema, the heart became enlarged and the pulmonic second sound remained accentuated. No blood pressures were recorded.

DISCUSSION AND CLINICAL CONSIDERATIONS

The various circulatory changes resulting from the deflation of one lung are indicative of the compensatory ability of the heart to respond to the heightened blood pressure and greater resistence created in the pulmonary circulation and to the deoxygenation occasioned by the collapse of the lung. These changes, when studied carefully in connection with pneumothorax therapy, are of a certain value in regulating the intervals and amounts of gas to be inflated into the pleural cavity, and prove also of assistance in the prognostication of the case.

In my article, *The Classification of Artificial Pneumothorax and the Clinical Value of the Several Types* (1), I indicated that an enlargement of the heart takes place in every case treated with pneumothorax, provided the reserve energy of the myocardium has not yet been exhausted and it is able to come into action whenever demand is made upon it. The right ventricle drives venous blood at a comparatively low pressure through the pulmonary vessels. These vessels play a relatively subordinate part in controlling the circulation in the lungs, for the pulmonary arteries possess little, if any, tone. Whereas, in the greater circulation the degree of constriction of the blood vessels depends on the condition of the smooth muscle fibres of the arteries and on the vasomotor nerves which supply them, in the lesser circulation it depends primarily on the condition of the lungs. When the alveoli are overdistended and their walls thinned out, thus constricting the capillaries, the resistance in the pulmonary flow is increased and the heart becomes hypertrophied. The same thing happens when the alveoli and their capillaries are compressed, as in pleural effusion or pneumothorax, particularly when the intrapleural pressure supersedes the pulmonary intravascular tension. Consequently, the more we deflate the lung with artificial pneumothorax the more we constrict the pulmonary vessels, augmenting thereby the blood pressure, not only in the compressed lung, but also in the contralateral one, through which a greater volume of blood has to flow per unit of time, in order to maintain an equilibrium between the lesser and greater circulation.

Schlaepfer (2), experimenting with ligation of the pulmonary artery in various animals, found that the pressure in the remaining pulmonary artery rose about 40 per cent, and that the right heart pumped out the same amount of blood with each contraction after ligation. This insures a normal arterialization of the blood by the one functioning lung. In

artificial pneumothorax, however, the pulmonary circulation is not entirely diverted to the noncollapsed lung, but continues to flow also through the deflated lung, depending, of course, on the degree of compression to which this lung is subjected. The larger the insufflated amounts and the higher the intrapleural pressure, the more are the pulmonary vessels compressed and greater the resistance they offer to the blood-flow in the lesser circulation. However, if the right heart is in good condition, it is able to meet this increased demand made upon it to drive the blood against an increased resistance in the pulmonary circulation.

Clinically, this is manifested by the accentuation of the pulmonic second sound, which is usually present in such cases. If, on the other hand, the reserve power of the heart has been exhausted by the long-continued toxemia, the right ventricle fails to compensate and is unable to meet the increased task put upon it by the elevated pulmonary pressure. In such cases the pulmonic sound is seldom accentuated. These patients are very prone to dyspnea, and their vital capacity is at a very low ebb.

In discussing the various factors modifying the vital capacity in artificial pneumothorax (3), I pointed out that the compensatory ability of the heart plays a great rôle, and with the failure of this cardiac compensation there occurs a great diminution of the vital capacity of these patients. The amount of gas injected into the pleural cavity of such patients should never exceed one-sixth of their vital capacity; otherwise dyspnea ensues.

Underhill (4), in his investigation of the circulation through the lungs of animals, found that whenever he ligated one of the pulmonary arteries there occurred a rise in the carotid blood pressure. Occasionally the carotid blood pressure showed a fall after ligation of the pulmonic artery, "but this was only seen in animals in poor condition, and indicated that the heart was unequal to the strain put upon it." The same phenomena were observed in the cases treated with artificial pneumothorax; those who showed a temporary rise in the systemic blood pressure after the first few inflations were young adults, who gave a history of pulmonary tuberculosis of short duration and were in fair general condition, whereas those which showed a fall or no change in blood pressure after the induction of pneumothorax had been sick for many years and were in poor condition at the initiation of the treatment.

The reason for the rise in the systemic blood pressure after the deflation

of one lung is probably the deficient oxygenation of the blood caused by this condition. Underhill (4) found that when he used excessive artificial ventilation of the lungs, after ligating one of the pulmonic arteries, there was no rise in the carotid pressure, and he concluded therefrom that the rise in the systemic blood pressure was due to the reduced lung ventilation, which occasioned a deficient oxygenation of the blood. Dock and Harrison (5), studying the blood flow through the lung in experimental pneumothorax, found that there occurred a marked decrease in the arterial oxygen only during the period of time shortly after initial collapse. This no doubt holds true also in clinical pneumothorax, and Underhill's explanation that the rise in the systemic blood pressure is due to deficient oxygenation seems very plausible. This is another reason why the initial amounts of the inflations should be very small, in order to avoid a sudden, marked reduction in the oxygen of the arteries. A fall in the systemic blood pressure after the induction of pneumothorax is a particular warning against large insufflated amounts, because it signifies a damaged compensatory mechanism of the heart failing to perform the additional work put upon it by the altered pneumodynamics. Such cases show very little tendency to the development of compensatory emphysema, which is of great importance in the course of pneumothorax therapy.

A clear distinction between hypertrophic emphysema and compensatory emphysema should always be kept in mind. In hyperthropic emphysema the pulmonary capillaries are compressed because of overdistention of the alveoli, the elastic tissue of which has lost a great deal of its contractile power, the residual air is increased, and the ventilation of the lungs is very poor. In compensatory emphysema, on the contrary, the extension of alveoli is the result of engorgement and dilatation of the capillaries surrounding the air sacs. V. Basch (6) has proved that coils of rubber tubing, filled with fluid and wound around a rubber bag, cause an expansion of the bag when the fluid contents in the tube is increased. Schlaepfer (2) obtained an enlargement of the alveoli by injecting bismuth-gelatine suspension into the pulmonary artery of the lung, and in his experiment with ligation of one pulmonary artery he noticed a marked compensatory hypertrophy of the intact lung, which pushed the mediastinum and the heart toward the operated side. The elastic tissue of such a vicariously hypertrophied lung retains both its expansile and contractile power; consequently the lung ventilation is more effective.

The development of compensatory emphysema should be looked upon as a very favorable sign, and it develops not only in the contralateral lung but also in the functioning portion of the collapsed lung in cases treated with pneumothorax of the expansile type. However, if the heart fails to adapt itself to the altered pneumodynamics, no compensatory emphysema develops and dypsnea is usually present. Such cases should never be given large amounts of gas at one treatment, but are better inflated with small quantities at frequent intervals.

The slight blood changes noticed after the institution of pneumothorax might probably also be considered as an attempt at compensation for the deficient oxygenation produced by the collapse of one lung, but our data in this respect were rather scanty and no definite conclusions could be drawn from them.

CONCLUSIONS

The circulatory changes resulting from the deflation of one lung can be divided into two groups:

1. Cases with an adequate reserve power of the heart which is able to adapt itself to the altered pneumodynamics: such cases usually show an increase in the systemic blood pressure after the first few inhalations, the pulmonic second sound becomes accentuated, compensatory emphysema develops in the functioning portion of the lungs, and the heart becomes enlarged in the course of the treatment. All these signs, jointly or singly, indicate a favorable prognosis.

2. Cases in poor condition, the cardiac reserve power of which has become exhausted by the long continued toxemia and its compensatory mechanism has been damaged: such cases rarely develop vicarious emphysema, the heart does not hypertrophy, the pulmonic second sound is seldom accentuated, and the vital capacity reduction is marked and is associated with dyspnea. The ultimate result of the treatment in such cases is not very successful.

The lungs should never be collapsed too rapidly; but the insufflations are to be made frequently and in very small quantities, in order to avoid a too sudden deoxygenation of the arterial blood. In connection with the symptomatology, roentgenology and spirometry, close observations of the circulatory changes before and after the introduction of pneumothorax are of help in the regulation of the insufflated amounts and in the prognostication of the case.

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TUBERCLE-BACILLUS SEPTICEMIA IN A HORSE CAUSED BY BACILLI OF AVIAN ORIGIN[1]

E. A. WATSON AND L. M. HEATH

The term *tubercle-bacillus septicemia* is used in this paper to denote a form of bacillary infection, so acute, so intense and so diffuse as to cause a fatal termination before any apparent localization of infection or development of miliary tubercles had taken place.

We have searched the literature on tuberculosis without finding a single record of a case of this kind in a horse, either occurring naturally or produced by experimental infection.

During the course of experiments which we have undertaken for studying in different animal species the pathogenicity of and the resistance to different strains of tubercle bacilli of human, bovine and avian type and origin (experiments which are still continuing), there developed in a horse inoculated with avian tubercle bacilli the acute form of tubercle-bacillus septicemia as defined above.

This was the more remarkable, as among the other horses inoculated with exactly equivalent amounts of bacilli of human and bovine type and origin, at the same time and in precisely the same manner, not one developed a bacillemic or septicemic form of infection. This apparently unique experience seems to us of such exceptional interest as to justify placing it on record now, separating it from the other experiments, of which it was originally a part and of which accounts will be published in full at later dates.

The infrequency of tuberculosis in the horse, as compared with cattle, swine and poultry, probably accounts for the fact that the isolation and study of tubercle bacilli of equine origin have been conducted mainly in connection with attempts to establish a relationship to the so called human, bovine and avian types of bacilli. The evidence produced shows that both natural and artificial infections of horses may be caused by bacilli conforming to either of these three types, and by bacilli of apparently intermediary types, the bovine type being the more common in

[1] From the Pathological Division, Health of Animals Branch, Department of Agriculture, Canada.

nature. Very few attempts, however, seem to have been made to infect horses by inoculation of tuberculous material obtained direct from infected fowls or with freshly isolated cultures of avian bacilli.

McFadyean in 1918 (*Jour. Comp. Path. and Therap., 225*) recorded a case of tuberculosis in a horse, remarkable, he states, "both on account of the peculiar nature of the lesions and because the bacilli found in these were proved to conform to what is termed the avian type of tubercle bacillus." In the same paper the author gives a summary of previously published cases of equine tuberculosis, in which the type of the causal bacilli was ascertained, also of previously published attempts to infect horses with artificial cultures of avian tubercle bacilli (Nocard, 1894–96; the British Royal Commission on Tuberculosis, 1911; Zwick and Zeller, 1913; Titze, 1906; Bang, 1909). We will not encumber this paper by again reviewing the literature cited, stopping only to mention briefly the results of the recorded attempts to infect horses with cultures of avian bacilli. Thus:

The British Royal Commission on Tuberculosis experimented on five ponies:

(1) A pony inoculated intravenously with 10 mgm. of culture died in 15 days. The lungs were heavy, congested and oedematous; the spleen was swollen and dark red. Tubercle bacilli were only moderately numerous in a smear preparation made from the lung, and scanty in the smears made from the liver, spleen, kidney and prescapular and mesenteric lymph nodes.

(2) A pony inoculated subcutaneously with 100 mgm. of culture died after 38 days, death being due to "acute septic peritonitis and pyaemia," of which the cause could not be ascertained.

(3) A pony inoculated subcutaneously with 50 mgm. of culture was killed when well, after 158 days. A very few tuberculous nodules were found in the lungs and prescapular lymph nodes (one tubercle bacillus found in a smear).

(4) A pony, given by mouth 100 mgm. of culture and killed after 160 days, showed only two minute foci in the small intestine and one in a mesenteric lymph node. No tubercle bacilli were found.

(5) A pony was fed for 34 days with cultures amounting to 600 mgm., and was killed when in apparent health after 138 days. Tuberculous lesions were found in many of the lymph nodes, and in the lungs, liver and omentum. Tubercle bacilli were present in the right tonsil, the cervical and mesenteric lymph nodes and in the omentum, but none was found in a smear from the liver tubercles.

Titze attempted to infect a six-months-old foal by feeding it with the growth of 44 culture tubes of avian bacilli, spread over a period of nearly three months, without success. When killed nine months later, no lesions could be found.

Bang fed two horses and a foal with cultures of avian bacilli. Both the horses, when killed after eight months, were found to be completely free from tuberculosis. The foal died 52 days after it had been fed 100 gm. of broth culture. Lesions were found in the mesenteric lymph nodes and in the solitary follicles of the intestine. Tubercle bacilli were numerous in these lesions.

Calmette, in his *Tubercle Bacillus Infection and Tuberculosis* (English Edition), deals at length with the virulence of mammalian bacilli for birds and that of avian bacilli for mammals, but as regards the latter for horses confines himself to the statement, "In horses, intravenous injection of avian cultures is very virulent and a generalized tuberculous process develops with more or less rapidity according to the dose." No details or references are given.

Neither in the foregoing, nor, so far as we are aware, in any other references or publications, is there any mention made of an acute tubercle-bacillus septicemia in a horse resulting either from a natural or artificial infection.

In our experiments an avian strain of tubercle bacillus (laboratory no. 101) was used, of which the details of origin, cultivation, preparation of suspensions, etc., are as follows:

Origin and Source: A flock of birds on a farm in the vicinity of Ottawa among which tuberculosis was causing havoc. Postmortem examination of fowls, which had died of the disease, showed typical pictures of avian tuberculosis. From this farm three live birds, in an emaciated condition, were sent to the laboratory. One of these, killed on January 5, 1924, was the source of the strain isolated. The other two birds were subjected to intracutaneous tests with bovine and avian tuberculin, injected simultaneously, the bovine into one wattle and the avian into the other wattle. Both birds reacted to both tuberculins, the reactions to the avian preparation being the stronger. The lesions in all three birds were very extensive and typical of generalized avian tuberculosis. On this farm 19 cattle in a herd of 30 to 35 animals also reacted to tuberculin at the first test.

Isolation and Culture: Primary growths were obtained on Dorset's egg media and on agar prepared with potato water, on which fragments of tuberculous liver had been spread, January 5, 1924. Subcultures were made on February 20th, on nutrient agar, $2\frac{1}{2}$ per cent glycerine and 1 per cent sugar, and continued on this media. On Dorset's media the bacilli were quite short, on glycerinated potato-water agar they were much longer and more deeply acid-fast, and in the older cultures granules and beaded forms could be noted.

Method of Preparing Bacillary Suspension for Inoculation: 100 mgm., moist weight, of the surface growth from solid culture media was thoroughly ground into a paste and slowly incorporated in 100 cc. of normal salt solution, and then shaken with glass beads in a mechanical shaker. The stock suspension, thus prepared, containing 1 mgm. of bacilli per cubic centimetre, can be diluted according to the dosage requirements and permits of a fairly accurate system of measurement.

Animals Artificially Injected: Horse (no. 16). This animal, a mare, eight years old, was born and raised at the Research Station, had never been off the premises, nor in a stable, nor in contact with any tuberculous animal or natural source of infection. It had always lived in the open, and had never been handled until halter-broken a month before inoculation. It was one of a group of six horses subjected at the same time to pathogenicity tests of six different strains of tubercle bacilli of the human, bovine and avian types, and was the first animal in the group to develop symptoms of disease and to succumb to infection. Before inoculation an intracutaneous tuberculin and complement-fixation tests were made, all with negative results.

Inoculations were made as follows:

July 14, 1924, intravenously, 1 mgm. of bacilli, 91 days' culture, 3rd generation.

July 24, 1924, intravenously, 2 mgm. of bacilli, 101 days' culture, 3rd generation.

August 7, 1924, intravenously, 5 mgm. of bacilli, 29 days' culture, 4th generation.

At the time of the first inoculation and at four-day intervals thereafter, a sample of blood was taken for complement-fixation tests, in which a special preparation of bovine tuberculin was used as the antigen (it had proved a satisfactory antigen in testing and titrating sera of other tuberculous animals). The first seven tests, July 14 to August 7, were all negative. No clinical signs of change in health had been manifested and the body temperature remained normal. The third intravenous inoculation was then made. Two days later and continuing for three days, August 9 to 11, a thermal reaction, 102.4°F. was recorded, from August 15 to 21 a reaction fluctuating between 102.8° and 104.8°F., and from August 22 to 27 a continuous fever just above and below 105°F. Four days after this third inoculation, August 11, the first positive complement-fixation reaction (3 plus) was given, with 0.2 cc. of serum. On

August 15, the reaction was positive with 0.05 cc. and on August 19 with 0.1 cc., and was again negative on August 24, three days before death, which took place twenty days after the third, or forty-four days after the first inoculation.

Six days before death the mare showed marked distress and kept apart from the other five horses in the field. A dazed, semicomatose condition ensued. Three days later a swelling appeared along the floor of the abdomen extending forward to the pectoral region. The udder was also involved, giving the appearance of incipient lactation. The next day, the animal could walk only with the greatest difficulty, with incoordination of movement and with a staggering gait. The eyes had a worried expression and the eyelids seemed to have lost their reflex action, but there was no evidence of myopia. When in a quiescent, standing position, the forelegs were spread well apart and braced, and the head and neck were lowered and extended. A frothy discharge from the mouth and a sanguinary exudate from the right nostril, the day before death, completed a distressing clinical picture that is not easily forgotten. The mare died on August 27th.

Postmortem examination was made about 12 hours after death. The body was well nourished and still in fair condition. *Lungs:* Dark slate-gray in color, some areas darker than others. Infiltrated with serous amber-colored fluid which, with froth, exuded from cut surfaces. *Heart:* Enlarged haemorrhagic areas in the myocardium; auricles depressed; antemortem clots in ventricles. Blood dark in color and not clotted in veins. *Lymph nodes:* Bronchial and mediastinal nodes pale in color, with watery exudate on section. *Liver:* Pale, soft and friable. *Kidneys:* Very pale, of doughy consistence, pitting upon pressure; destruction of the cortical structure. *Spleen:* Twice normal size, and very dark in color, the splenic pulp suggesting a resemblance to black-currant jam. *Stomach:* Almost empty, a few bot larvae present. *Intestines:* Haemorrhages throughout. *Bladder:* Contains about two ounces of creamy liquid. There was no visible evidence in any of the organs or tissues of miliary tubercles or fine granules, nor any appearance suggestive of their presence.

Microscopic Examination: The material examined was taken from the liver, spleen, kidneys and lungs. Fresh impressions and smears taken from the cut-surfaces of any portion of these organs, stained by the Ziehl-Neelsen method, showed incredible numbers of tubercle bacilli in every microscopic field. The invasion seemed rather more dense in the

abdominal organs than in the lungs, and was densest perhaps in the spleen, but all specimens teemed with acid-fast bacilli to such an extent that it is difficult to draw a difference of degree. The impression preparations showed the bacilli, for the most part, packed in nests, or rounded colonies, varying in size from that of a small monocyte to a large epithelioid cell. These could easily be seen under the low power (16 mm.) objective, as numerous red, round, granular bodies of various sizes. Many of these colonies appeared to be breaking up into small masses and groups of bacilli scattered all through the preparation, giving the microscopic appearance of a young artificial culture mixed with some tissue cells. In these preparations the free cells were nearly all of the mononuclear varieties, polymorphonuclear leucocytes being rare. The liver and kidney cells showed marked cloudy swelling, vacuolation and disintegration.

Sections of the liver, spleen, kidneys and lungs were fixed in formalin, embedded in paraffin and stained by the Ziehl-Neelsen method, the Giemsa method and with haematoxylin and eosin. All sections of these organs showed a very marked infiltration of mononuclear cells, varying in size but for the most part having the appearance and character of epithelioid cells or endothelial leucocytes. In sections of the lungs the picture was that of the exudative form of acute tuberculous inflammation. The interalveolar connective tissues were invaded with monocytes, which also appeared in large numbers in the exudate which filled many of the alveolar spaces and bronchioles. Some of the alveoli showed a desquamation of the epithelial cells, but these were few in comparison with the great numbers of endothelial leucocytes.

The liver cells appeared swollen, and seemed to be separating from the columns of cells which were in marked disorder. Many of the cellular nuclei did not take the stain, others did so but faintly. A fairly heavy deposition of brown pigment granules had taken place.

The cells of the spleen pulp were swollen and degenerated, many of the nuclei disintegrating. There was a very heavy deposition of pigment in granular flakes and clumps.

In all sections of the lungs, liver, spleen and kidney, the nested, colony-like formation of the tubercle bacilli was very conspicuous. These nests appeared, for the most part, to occupy the large monocytes, the smaller nests being well defined in the protoplasm of the cell, the larger occupying the whole of the cell so that neither nucleus nor protoplasm could be observed. There were also numerous round or oval vacuoles, corre-

sponding in size, shape and location with the intracellular colonies. Some of the former were quite empty, while others contained tubercle bacilli, arrayed in small clumps or as a marginal ring surrounding the empty cell or vacuole.

Neither giant cells nor minute miliary tubercles could be definitely discerned.

LABORATORY ANIMALS

The virulence and pathogenicity of the identical cultures and bacillary suspensions with which the horse was infected were determined for guinea pigs and rabbits.

1. On July 14th, simultaneously with the first inoculation of the horse, two guinea pigs were inoculated:

Guinea pig (no. 9) received 0.5 mgm. bacilli subcutaneously. It was apparently in normal health and condition when killed 191 days later. An enlargement of an iliac lymph node was noted. No tubercle bacilli were seen in smear preparations.

Guinea pig (no. 10) received 0.5 mgm. bacilli intraperitoneally. It died 56 days later. There were emaciation and pneumonia. The spleen was enlarged, and the mesenteric lymph nodes were caseocalcareous. A few tubercle bacilli were seen in smear preparations.

2. On July 24th, simultaneously with the second inoculation of the horse, one rabbit and two guinea pigs were inoculated:

Rabbit (no. 4) received 0.5 mgm. bacilli intravenously, and died 19 days later from an acute bacillemic infection. There were no visible tubercles, either miliary or granular, but the spleen was greatly enlarged and the organs were teeming with acid-fast bacilli.

Guinea pig (no. 17) received 0.5 mgm. of bacilli subcutaneously. Apparently in normal health and condition 181 days later, when killed. An enlarged iliac lymph node was the only lesion noted, and bacilli were found in the smear preparation.

Guinea pig (no. 18) received 0.5 mgm. of bacilli intraperitoneally, and was killed 181 days later. Slight lesions of the spleen and mesenteric lymph nodes. Bacilli found in smear preparations.

3. On August 7th, simultaneously with the third inoculation of the horse, one rabbit and two guinea pigs were inoculated:

Rabbit (no. 9) received 0.5 mgm. of bacilli intravenously, and died

in 24 days from an acute bacillemic infection. No visible tubercles, miliary or granular. Spleen greatly enlarged, and teeming with acid-fast bacilli.

Guinea pig (no. 27) received 0.5 mgm. of bacilli subcutaneously and was killed 167 days later. Tuberculous lesions in spleen and hepatic lymph node.

Guinea pig (no. 28) received 0.5 mgm. of bacilli intraperitoneally, and died in 26 days; only the mesenteric lymph nodes appeared tuberculous.

4. On August 28th, one rabbit, two guinea pigs and six white rats were infected with a suspension of spleen pulp of the horse which died of tubercle-bacillus septicemia:

Rabbit (no. 13) received 0.5 cc. of the spleen-pulp suspension intravenously, and died in 26 days, with spleen greatly enlarged and teeming with acid-fast bacilli.

Guinea pig (no. 35) received 0.5 cc. intraperitoneally and died in 19 days. The liver showed pin-point lesions; the spleen was enlarged. Acid-fast bacilli in smear preparations.

Guinea pig (no. 36) received 0.5 cc. subcutaneously, and was killed 150 days later. Condition normal. No lesions.

White rat (no. 1) received 0.5 cc. intraperitoneally. Killed 116 days later. No lesions.

White rat (no. 2) received 0.5 cc. subcutaneously. Killed 116 days later. In an abscess, which developed at the site of inoculation, large numbers of acid-fast bacilli were present. No other lesions.

White rats (nos. 3, 4, 5 and 6), were fed with spleen and liver of horse no. 16, and were killed 116 days later. No lesions.

Summary of Tests on Laboratory Animals: The three rabbits died in 19, 24 and 26 days, respectively, each from a bacillemic or septicemic type of infection similar to that in the horse. The guinea pigs, for the most part, were highly resistant to infection. Three animals died a natural death in 19, 26 and 56 days, respectively, which it is hard to attribute to tuberculosis, as the lesions were apparently slight and the bacilli present in scanty numbers. The other five animals were killed after five or six months; slight or suspicious lesions were noted in four, and acid-fast bacilli were found in three of these animals. Inoculation into white rats was negative in one animal and resulted in a local infection in the other. Four rats appeared immune to infection by ingestion.

THE AGGLUTINATION OF TUBERCLE BACILLI[1]

J. FREUND

The mechanism of the agglutination of bacteria, especially the relation between agglutination, difference of potential and cohesive force, has been established by recent studies. The studies were carried out on *Bacillus typhosus* and the bacillus of rabbit septicemia, two gram-negative bacteria. One of the main results of the studies mentioned is the fact that the behavior of these bacteria closely resembles that of protein particles in suspension. It appears to be of interest to take up similar studies on tubercle bacilli, as their chemical composition is so markedly different from that of the gram-negative bacteria. Such studies may throw some light on the chemical nature of the surface of the bacteria, and also on the serological aspects of the agglutination of the tubercle bacillus.

We can summarize the results of recent studies on agglutination as follows: The precipitation of protein particles and the agglutination of a bacterial suspension depend on two forces. One of them is the repulsive force which tends to keep the particles apart; the other is the cohesive force which tends to bring them together. The repulsive force is the result of the electrical charge of the particles, which can be measured by the rate of migration of the particles in an electrical field (1). There is but little known about the nature of the cohesive force; yet it can be measured by a method devised by Northrop and DeKruif. Their work has shown that the potential of *Bacillus typhosus* and of bacillus of rabbit septicemia, washed and suspended in distilled water, is considerably higher than 15 millivolts, and that the bacteria are agglutinated when the potential is reduced below 15 millivolts if the cohesive force is not affected. Electrolytes in concentration less than 0.01 molecular do not affect the cohesive force. Electrolytes in higher concentration decrease the cohesive force. In this case the critical potential for the agglutination is also decreased, and in concentrated electrolyte solutions agglutination does not occur in spite of the very low potential. In agglutination by immune serum "the immune serum prevents the salts from

[1] From the von Ruck Research Laboratory for Tuberculosis, Asheville, North Carolina.

decreasing the cohesive force between the organisms; therefore, agglutination is determined solely by the potential." Accordingly, agglutination occurs even in the presence of electrolytes in concentration above 0.01 molecular.

The present study deals with the agglutination of tubercle bacilli and its constituents by electrolytes and by immune serum.

EXPERIMENTAL

Two strains of tubercle bacilli were studied, strains H597 and HD. Strain H597 is an old laboratory strain, virulent for guinea pigs, and it grows rapidly enough to cover the surface of 200 cc. of broth in two days. Otherwise, it is a typical strain of *Bacillus tuberculosis*. The strain HD is a typical strain of *Bacillus tuberculosis* of human type, and of medium virulence.

In order to provide a homogeneous suspension, strain H597 was grown for three days on glycerol-broth, and then filtered and washed three times with distilled water. The washed bacilli were ground with distilled water for half an hour to two hours, and then centrifugated. The supernatant suspension was used. The suspension, stained according to Ziehl-Neelsen, showed acid-fast bacilli, and here and there acid-fast clumps. Suspensions of strain HD were made from a culture about 3 to 4 weeks old, and prepared exactly like strain H597. Old cultures are not well adapted for making homogeneous suspensions.[2] The grinding was done mechanically with marbles of about 5 gm. Usually 2 to 3 drops of the suspension were added to 25 cc. of an electrolyte solution. The potential of the bacteria was measured by a microscopic cataphoresis cell devised by Northrop (1). The potential was measured immediately after the preparation was made up, and the reading of the agglutination was done about twenty hours later (room temperature).

\+ means agglutination noticed by using the lens only.
\+\+ means agglutination noticed by the naked eye.
\+\+\+ means heavy agglutination, and supernatant fluid not clear.
c means complete agglutination, supernatant fluid clear.

[2] The method of Bossau and Roset (2) to obtain homogeneous cultures of tubercle bacilli by the continuous aeration of the medium was tried without satisfactory results.

AGGLUTINATION OF TUBERCLE BACILLI BY ELECTROLYTES

The effect of electrolytes on tubercle bacilli is shown in table 1. The potential of the bacteria is reduced by different electrolytes. Copper, cadmium, thorium, and hydrogen ions change the sign of the electrical charge. The higher the value of the ion, the greater is the reducing effect with the exception of hydrogen ion, which is the most effective. The electrolytes copper, cadmium, thorium, and hydrogen reduce the potential markedly in low concentration, and cause agglutination whenever the potential difference reaches about ± 12.5 millivolts; while sodium, potassium, calcium and magnesium, the reducing effect of which is less, do not cause agglutination in any concentration, as a rule.

It was observed that two cultures of HD out of five gave suspensions which were less stable toward the effect of calcium and magnesium. They showed agglutination at the concentrations 1/64, 1/32, and 1/16 molecular. The optimum agglutination was at 1/32 molecular, at which concentration the potential of the bacteria, as in all the suspensions used, was about -12.5 millivolts. The reason for this irregularity is very likely the quantitative difference in the cohesive forces of the different suspensions. In the case of most of the preparations, the concentration of calcium chloride or magnesium chloride, which is sufficient to decrease the potential difference to the critical value (12.5 millivolts), prevents agglutination by decreasing the cohesive force, while, in the case of suspensions showing this zone phenomenon, the cohesive force appears to be higher, so that only a higher concentration of calcium chloride and magnesium chloride prevents the agglutination.

The stabilizing effect of sodium chloride in higher concentration than 1/3 molecular and of magnesium chloride in higher concentration than 1/32 molecular in the case of streptococci, which could not be suspended even in distilled water, has been described by Mellon and his co-workers (3).

AGGLUTINATION OF DEFATTED TUBERCLE BACILLI

Originally the surface of tubercle bacilli was thought to be made up of lipoids. This assumption was made to explain their acid-fastness and their great resistance toward chemical agents. Subsequently this explanation was dropped because of the observation that thoroughly ground bacilli lose their acid-fastness (Koch). Long suggests that the acid-fastness is probably due to the physical state of the lipoid-protein mixture of the bacteria (4).

TABLE I

Effect of electrolytes on potential difference and agglutination of tubercle bacilli*

NaCl			$CaCl_2$			$CaCl_2$		
Concentration of NaCl	Potential difference	Agglutination	Concentration of $CaCl_2$	Potential difference	Agglutination	Concentration of $CaCl_2$	Potential difference	Agglutination
mol/litre			*mol/litre*			*mol/litre*		
M/2	−9.2	−	M/5	−4	−	M/10	+ very low	c
M/4	−12	−	M/20	−9.2	−	M/20	+ very low	c
M/8	−18	−	M/40	−9.8	−	M/40	− very low	c
M/16	−25	−	M/80	−11	−	M/80	− very low	c
M/32	−27	−	M/160	−11.2	−	M/160	−5.2	c
M/150	−28.7	−	M/320	−12	−	M/320	−10	c
M/600	−36.5	−	M/1280	−17.5	−	M/640	−11.7	c
M/2500	−39	−	M/2560	−22.5	−	M/1280	−12.1	−
M/10000	−40.5	−	Distilled water	−55	−	M/2560	−16.2	−
Distilled water	−40	−				M/5120	−19	−
						M/10240	−25.5	−
						M/20480	−31	−
						M/81920	−43	−
						Distilled water	−43.5	−
$CuSO_4$			$Th(NO_3)_4$			HCl		
M/20	+ very low	c	M/1000	+30	−	M/800	+5	c (pH = 2.8)
M/100	− very low	c	M/4000	+28.3	−	M/1600	+ very low	c (pH = 3.3)
M/200	− very low	c	M/8000	+12.7	c	M/3200	−10.5	c (pH = 3.8)
M/400	− very low	c	M/16000	−32.7	−	M/6400	−23	−
M/800	−4	c	M/32000	−37	−	M/12800	−30	−
M/1600	−6.1	c	M/64000	−39.2	−	M/25600	−35	−
M/3200	−10	c	M/128000	−41	−	M/51200	−36	−
M/6400	−15	−	Distilled water	−44	−	Distilled water	−40	−
M/12800	−21.2	−						
M/25600	−25	−						
M/51200	−31	−						
M/102400	−32.5	−						
Distilled water	−40	−						

*The potential difference is expressed in millivolts.

The comparative study of the behavior of tubercle bacilli and their constituents, namely, protein and lipoid toward the effect of electrolytes may throw some further light upon the question of the chemical composition of the surface.

Young cultures of strains H597 and HD were filtered, washed several times, and dried over sulphuric acid *in vacuo*. Then they were extracted successively with water-free ether, water-free alcohol and water-free chloroform for several days at room temperature, while being ground.

TABLE 2

Effect of electrolytes on potential difference and agglutination of defatted tubercle bacilli

CONCENTRATION OF SALTS	NaCl	$CaCl_2$	$CuSO_4$	
	Agglutination	Agglutination	Potential difference	Agglutination
mol/litre				
M	—	c		c
M/2	—	—		c
M/4	—	—		c
M/8	—	—		c
M/20	—	—		c
M/40	—	—		c
M/80	—	—		c
M/160	—	—		c
M/320	—	—		c
M/640	—	—		c
M/1280			— very low	c
M/2560			−8	c
M/4000			−14	+++
M/5120				+
M/8000			−21.5	—
M/16000			−28.5	—
Distilled water	—	—	−51	—

The bacilli thus defatted were not acid-fast. A suspension was made by grinding them with distilled water. Their behavior toward electrolytes is shown in table 2.

Sodium chloride and calcium chloride do not agglutinate the particles at concentrations from 1/1 to 1/16,000 molecular, although the charge of the particles is considerably reduced. Copper sulphate produces agglutination in higher concentrations than 1/5000 molecular when the potential is reduced below about −14 millivolts. Similar behavior was observed in suspensions of whole tubercle bacilli.

AGGLUTINATION OF THE LIPOIDS OF TUBERCLE BACILLI BY ELECTROLYTES

In order to prepare a suspension of lipoid particles, an alcoholic extract of tubercle bacilli was mixed with distilled water, and the alcohol was evaporated at about 30°C. The suspension was centrifugated and preserved with thymol. The stability of this suspension remained unchanged for about two months, and later it became even more stable. One can obtain suspensions of lipoids from alcoholic extracts by evaporating the alcohol at a low temperature and suspending the lipoid by grinding in distilled water. Thus one can obtain suspensions of different stability, but in using them for agglutination essentially the same results were obtained. Agglutination does not occur, or does not go so far, when the density of the lipoid suspension in the salts is too low. The lowest limit of density which can be used has to be determined empirically for every suspension. If one evaporates the alcohol at a higher temperature, for instance by boiling the solution, the lipoid cannot be suspended in water.

TABLE 3

Effect of electrolytes on potential difference and agglutination of lipoids of tubercle bacilli

CONCENTRATION OF SALTS	NaCl		$CaCl_2$		$CuSO_4$	
	Potential difference	Agglutination	Potential difference	Agglutination	Potential difference	Agglutination
mol/liter						
M	−9.2	c		c		c
M/2	−14	c		c		c
M/4	−19	—		c		c
M/8	−25.5	—		c		c
M/16		—		c		c
M/32		—	−8.5	c		c
M/50			−8.5	c		
M/64			−14.5	c		c
M/100			−13			
M/128			−14.5	c	−10	c
M/200			−16.2	—		
M/256				—		c
M/512				—	−15	c
M/1024				—		—
Distilled water	−46.5	—	−45	—	−45	—

Table 3 shows that sodium chloride agglutinates the suspension used at the concentrations 1/1 and 1/2 molecular, calcium chloride at con-

centrations from 1/1 to 1/128 molecular, and copper sulphate at concentrations from 1/1 to 1/500 molecular. The agglutination depends solely upon the potential. It occurs whenever the potential is reduced below about 15 millivolts, which is not the case either with whole tubercle bacilli or with the defatted particles in suspension. The higher concentrations (over 1/100 molecular) of sodium chloride and calcium chloride do not stabilize the lipoid particles, as in the case of the whole and the defatted bacteria. This suggests that the cohesive force between the lipoidal particles is not lowered, or not lowered enough to prevent agglutination.

The stability of the suspension, as mentioned above, was increased after the suspension had stood for two months. Originally it was agglutinated by sodium chloride at concentrations 1/1 and 1/2 molecular, by calcium chloride at concentrations from 1/1 to 1/128 molecular, and by copper sulphate at concentrations from 1/1 to 1/500 molecular. After two months it was agglutinated by sodium chloride at concentrations 1/1 and 1/2 molecular, by calcium chloride from 1/1 to 1/40 molecular, and by copper sulphate from 1/1 to 1/160 molecular.

AGGLUTINATION OF TUBERCLE BACILLI BY IMMUNE SERUM

In the agglutination of *Bacillus typhosus* and bacillus of rabbit septicemia, two effects of the serum were demonstrated by Northrop and DeKruif. Immune serum, as well as normal serum, reduces the actual potential of the bacteria when the concentration of the serum present is relatively high—higher than 1/100. Another effect of the immune serum which is specific is that "it prevents the salts from decreasing the cohesive force between the organisms:" therefore, agglutination is determined solely by the potential. As the titres of the immune sera available were low, in one case 1 to 40, in the other case 1 to 80, we could not avoid using high concentrations of sera. In order to remove the nonspecific influence of the sera, sensitized bacteria were used. To a dense suspension of bacteria in distilled water a large excess of immune serum was added (about 5 units). Immediately after the addition of serum, the suspension was centrifugated and washed once with distilled water. Rabbit immune sera were obtained from rabbits treated with killed suspensions of H597 and HD, respectively. Normal and immune sera were inactivated and preserved with phenol.

An account is given in table 4 of the effect of normal and immune serum

on the potential and agglutination of tubercle bacilli. The dilution of the serum was made with distilled water, so that the concentration of the salts present is negligible. The immune serum as well as the normal serum decreases the potential and causes slight agglutination. Apparently the immune serum is somewhat more effective in both respects than the normal serum. Agglutination occurs when the potential is low,—less than 8 millivolts. It is noteworthy that the critical potential for the agglutination in the presence of relatively high concentration of the serum is lower than it is without serum. This phenomenon shows a stabilizing property of the serum. This stabilizing effect is responsible

TABLE 4

Effect of normal and immune serum on potential difference and agglutination of tubercle bacilli

DILUTION OF SERUM	NORMAL SERUM		IMMUNE SERUM	
	Diluted with distilled water			
	Potential difference	Agglutination	Potential difference	Agglutination
1/5	−8.5	−	−2.5	+++
1/10	−10	−	−8.5	++
1/20	−13.1	−	−11.3	+
1/40	−16.8	−	−12.9	−
1/80	−19.1	−	−21.3	−
1/160	−22.3	−	−28	−
1/320	−23.5	−	−29.8	−
1/640	−35	−	−36.5	−
1/1280	−37.5	−	−36.5	−
Distilled water	−37.2	−	−37.2	−

for the fact that immune and normal serum agglutination, when the sera are diluted with 0.9 per cent sodium-chloride solution, fails to appear, or is very weak, in the tubes containing undiluted and slightly diluted serum (1 to 2, 1 to 4).

The specific effect of immune serum was demonstrated with sensitized and washed bacteria as described above. Table 5 shows that agglutination occurs whenever the potential is reduced below about −13.0 millivolts, even in calcium-chloride and sodium-chloride solutions. The sensitizing prevents the salts from decreasing the cohesive force between the organisms.

TABLE 5

Effect of electrolytes on the potential difference and agglutination of sensitized tubercle bacilli

NORMAL SERUM			IMMUNE SERUM							
Concentration of salts	$CuSO_4$		Concentration of salts	NaCl		$CaCl_2$		$CuSO_4$		$Th(NO_3)_4$	
	Potential difference	Agglutination		Potential difference	Agglutination	Potential difference	Agglutination	Potential difference	Agglutination	Potential difference	Agglutination
mol/litre			*mol/litre*								
M/1000	0	c	M/2	almost 0	−	0	−				
M/2000	very low	c	M/4	almost 0	−	0	−				
M/4000	−3.5	c	M/8	−4.5	+	0	+				
M/8000	−8.8	c	M/16	−7	c	0	c				
M/10000			M/32	−10	c	− very low	c				
M/20000	−18.2	−	M/64	−12.7	+	− very low	+++				
M/40000	−24	−	M/128	−16.5	−	− very low	+++				
M/8000	−33	−	M/256	−18.7	−	−7	+++				
Distilled water	−34.5	−	M/512			−8.2	+++				
			M/1024			−12.5	+++	−very low	c	+22	−
			M/2048			−15	−	− very low	c		
			M/4096				−	−7	c	+17	−
			M/8192					−11.2	c		
			M/16394					−14.2	−	+7	c
			M/32788					−19.5	−	−8	c
			Distilled water	−25	−	−29	−	−28	−	−29	−

AGGLUTINATION OF DEFATTED TUBERCLE BACILLI BY NORMAL AND IMMUNE SERUM

A suspension of defatted tubercle bacilli was made as described above. The suspension used was not agglutinated by sodium chloride and by calcium chloride. It was agglutinated by copper sulphate at concentrations from 1/1 to 1/1000 molecular.

First it was shown that the suspension of defatted bacilli was agglutinated by immune serum, using the routine method for agglutination. Table 6 shows comparatively that immune serum agglutinates the particles in dilution 1 to 16 completely and at 1 to 64 incompletely, while a

TABLE 6

Effect of normal and immune serum on the agglutination of defatted tubercle bacilli

DILUTION OF SERUM	NORMAL SERUM	IMMUNE SERUM
	Diluted with physiological NaCl	
1/1	+	+++
1/2	+	c
1/4	+	c
1/8	+++	c
1/16	−	c
1/32	−	+++
1/64	−	++
1/128	−	−
1/256	−	−
1/512	−	−
1/1024	−	−
1/2048	−	−
Distilled water	−	−

The used protein suspension was not agglutinated by NaCl and $CaCl_2$. By $CuSO_4$ it was agglutinated at concentrations of from M to M/1000.

strong but incomplete agglutination occurs with normal serum in dilution 1 to 8.

The defatted bacilli were treated with normal and immune serum as in the case of whole tubercle bacilli.

Table 7 shows that the defatted bacilli treated with normal serum were not agglutinated by sodium chloride; they were agglutinated by calcium chloride at concentrations from 1/1 to 1/500 molecular but incompletely; by copper sulphate they were agglutinated at concentrations from 1/2000 to 1/10000 molecular.

The defatted bacilli treated with immune serum were agglutinated by

sodium chloride at concentrations from 2/1 to 1/128 molecular, by calcium chloride at concentrations from 2/1 to 1/1000 molecular, and by copper sulphate at concentrations from 1/2000 to 1/10000 molecular.

These results show that the defatted bacilli behave like whole tubercle bacilli, except that the defatted bacilli treated with normal serum were agglutinated by calcium chloride, although incompletely, and whole bacilli treated with normal serum were nøt. The significance of this

TABLE 7

Agglutination of defatted, with normal and immune serum sensitized tubercle bacilli by electrolytes

CONCENTRATION OF SALTS	PARTICLES SENSITIZED WITH NORMAL SERUM			PARTICLES SENSITIZED WITH IMMUNE SERUM		
	NaCl	$CaCl_2$	$CuSO_4$	NaCl	$CaCl_2$	$CuSO_4$
mol/litre						
2M	−			+++	++	
M	−	++		+++	c	
M/2	−	++		+++	c	
M/4	−	++		c	c	
M/8	−	+++		c	c	
M/16	−	+++		c	c	
M/32	−	+++		c	c	
M/64	−	+++		c	c	
M/128	−	+++		c	c	
M/256	−	+++		−	c	
M/512	−	++		−	+++	
M/1024	−	−		−	+++	
M/2048	−	−	c	−	−	c
M/5096		−	c		−	c
M/10192		−	c		−	c
M/20384			−			−
M/40768			−			−
M/81536			−			−
Distilled water	−	−	−	−	−	−

difference is lessened by the fact that a few preparations of untreated bacillary suspensions were agglutinated by calcium chloride.

AGGLUTINATION OF LIPOID PARTICLES BY NORMAL AND IMMUNE SERUM

The lipoidal suspension used for the agglutination of electrolytes was employed.

First it was shown that the suspension of lipoid particles was agglutinated by immune serum in dilutions from 1 to 1:14, using the routine

method for agglutination (the serum diluted with 0.9 per cent sodium chloride). Table 8 shows that the presence of sodium chloride, even in small concentration, hinders the agglutination, although sodium chloride itself agglutinates the lipoid particles in concentrations of 1/1 and 1/2 molecular. Table 8 shows that the greatest difference between normal and immune serum can be obtained by making the dilution of the serum with distilled water. A control series showed that the addition of distilled water did not precipitate the serum in the dilutions used.

TABLE 8

Agglutination of lipoid particles by normal and immune serum

DILUTION OF SERUM	NORMAL SERUM						IMMUNE SERUM					
	Serum diluted with distilled water	Concentration of NaCl					Serum diluted with distilled water	Concentration of NaCl				
		M/30	M/15	M/7	M/4	M/2		M/30	M/15	M/7	M/4	M/2
1/1	−			−	−	−	c			c	c	c
1/2	c	c	+++	−	−	±	c		c	+++	+	+++
1/4	c	c	c	−	+	±	c	c	c	++	−	+
1/8	c	c	c	−	+	±	c	c	c	−	−	−
1/16	c	c	+++	−	+	−	c	c	c	−	−	−
1/32	c	c	+++	−	+	−	c	c	c	−	−	−
1/64	−	+++	−	−	−	−	c	c	c	−	−	−
1/128	−	−	−	−	−	−	c	+++	−	−	−	−
1/256	−	−	−	−	−	−	−	−	−	−	−	−
1/512	−	−	−	−	−	−	−	−	−	−	−	−
1/1024	−	−	−	−	−	−	−	−	−	−	−	−
1/2048	−	−	−	−	−	−	−	−	−	−	−	−
Control	−	−	−	−	−	−	−	−	−	−	−	−

Table 9 shows the relation between potential difference and agglutination. Both normal and immune serum decrease the potential of the lipoid particles, but in higher dilution the immune serum is more effective. Agglutination occurs in the case of normal serum only when the potential is reduced to 7 millivolts, while in the case of immune serum the critical potential is 12.7 millivolts. Similar observations concerning the decreasing effect on the actual potential by normal and immune serum was made on red blood cells by Northrop and Freund (5), and recently by Shibley on streptococci (6).

Electrolytes, as reported above (table 3), agglutinate a lipoid suspen-

sion whenever the potential of the particles is reduced below −15 millivolts. The comparison of table 3 and table 9 shows that not only the actual but also the critical potential for the agglutination is lowered by normal serum.

The agglutinating effect of the tuberculous immune serum on the suspension of lipoid particles appears to be specific, inasmuch as a suspen-

TABLE 9

Effect of normal and immune serum on agglutination and potential difference of lipoid particles

NORMAL SERUM			IMMUNE SERUM		
Diluted with distilled water					
Dilution of serum	Agglutination	Potential difference	Dilution of serum	Agglutination	Potential difference
1/20	c	−7	1/50	c	−10.5
1/40	−	−7.5	1/100	c	−12.2
1/80	−	−8.3	1/200	c	−12.7
1/160	−	−11.5	1/400	−	−13.3
1/320	−	−14.2	1/800	−	−15.2
1/640	−	−17.5	1/1600	−	−15.5

TABLE 10

Agglutination of lipoid particles prepared from staphylococci by normal serum and by tuberculous immune serum

DILUTION OF SERUM	NORMAL SERUM	TUBERCULOUS IMMUNE SERUM
	Diluted with distilled water	
1/2	++	++
1/4	c	c
1/8	c	c
1/16	c	c
1/32	−	−
1/64	−	−
1/128	−	−
Control	−	−

sion of lipoid particles prepared from staphylococci was not more strongly agglutinated by tuberculous immune serum than by normal serum (table 10). However, no observation was made with acid-fast saprophytes. Recent studies have shown that lipoids prepared from tubercle bacilli and other acid-fast bacteria give positive complement fixation with the serum of rabbits immunized with whole tubercle bacilli.

An effort was made to increase the difference between normal and

immune serum by applying calcium chloride and copper sulphate for the dilution of the serum. Table 11 shows that, although they increase the titre of normal and immune serum, they do not increase the difference between normal and immune serum. When using copper sulphate in the dilution of 1/16000 and 1/8000 molecular the titres of the normal and immune serum are the same. They differ only inasmuch as the zone of nonagglutination is smaller in the case of immune serum. These experi-

TABLE 11

*Agglutination of lipoid particles by normal and immune serum**

DILUTION OF SERUM	NORMAL SERUM			IMMUNE SERUM		
	Serum diluted with M/200 $CaCl_2$	Concentration of $CuSO_4$		Serum diluted with M/200 $CaCl_2$	Concentration of $CuSO_4$	
		M/16000	M/8000		M/16000	M/8000
1/2	+++	c	c	c	c	c
1/4	c	c	c	c	c	c
1/8	c	c	c	c	c	c
1/16	c	c	±	c	c	c
1/32	c	−	−	c	c	−
1/64	c	−	−	c	−	+++
1/128	c	−	−	c	−	c
1/256	+++	c	c	c	c	c
1/512	−	c	+++	+++	c	c
1/1024	−	c	c	++	c	c
1/2048	−	+++	c	+	c	+++
1/4096	−	−	−	−	+	−
1/8192		−	−	−	−	−
1/16384		−	−	−	−	−
1/32768		−	−	−	−	−
1/65536		−	−	−	−	−
1/131072		−	−	−	−	−
1/262144		−	−	−	−	−
1/524288		−	−	−	−	−
1/1948576		−	−	−	−	−
Control		−	−	−	−	−

* $CuSO_4$ precipitates the serum above the concentration M/4000.

ments were repeated several times with the same result. A similar phenomenon with a zone of nonagglutination in the case of bacterial suspension was reported by Bechold (7).

In order to exclude the nonspecific effect of the serum the agglutination of sensitized lipoid particles by electrolytes was observed. Lipoid particles were added to 5 cc. of serum diluted 1 to 5 with 0.9 per cent sodium chloride solution. The particles were centrifugated and resus-

pended in distilled water. Table 12 shows the effect of sodium chloride, calcium chloride and copper sulphate on the sensitized particles. It shows that the particles treated with normal serum are more sensitive than the untreated particles toward the electrolytes used, but the differences, except in the case of copper sulphate, are not considerable. Particles sensitized with immune serum are agglutinated by sodium chloride at the concentration 1/500 molecular (++), by calcium chloride at the

TABLE 12

Agglutination of sensitized lipoid particles by electrolytes

CONCENTRATION OF SALTS	NORMAL SERUM			IMMUNE SERUM			
	NaCl	$CaCl_2$	$CuSO_4$	NaCl	$CaCl_2$	$CaCl_2$ after 2 months	$CuSO_4$
mol/litre							
2M	c			c			
M	c	c		c	c	c	
M/2	+++	c		c	c	c	
M/4	+++	c		c	c	c	
M/8	+++	c		c	c	c	
M/16	+++	c		c	c	c	
M/32	+	c		c	c	c	
M/64	−	c		c	c	c	
M/128	−	c	c	c	c	c	
M/256	−	c	c	+++	c	c	
M/512	−	+++	c	++	c	c	
M/1024	−	+++	c	+	c	c	c
M/2048	−	−	+++	−	+++	c	c
M/4096	−	−	−	−	+++	c	c
M/8192	−	−	−	−	+++	−	c
M/16384			−		+++	−	c
M/32768			−		−	−	−
M/65536			−		−	−	−
M/131072			−		−	−	−
Distilled water	−	−	−	−	−	−	−

The agglutination by $CuSO_4$ was made after the lipoid particles had become as stable as shown at the electrolytic agglutination on page 126.

concentrations 1/16000 and 1/4000 molecular, and by copper sulphate at the concentration 1/16000 molecular. It is noteworthy that sodium chloride, which hinders the agglutination at very low salt concentration, has an agglutinating effect on untreated and sensitized lipoid particles.

The difference in the electrolyte agglutination between the particles treated with normal serum and those treated with immune serum demonstrates a specific agglutinating effect of the tuberculous immune serum on lipoids prepared from tubercle bacilli.

The quantitative relation between the agglutination, potential difference and cohesive force of sensitized lipoid particles, has not been established. Apparently the mechanism of agglutination in sensitized lipoid particles is quite different from that in the whole and defatted bacteria. In the latter cases the immune serum prevents the salts from decreasing the cohesive force between the sensitized particles, while even in untreated lipoidal particles the cohesive force is not diminished by the effect of the salts.

DETERMINATION OF ISOELECTRIC POINTS

In connection with this observation, the isoelectric points of tubercle bacilli, their protein and lipoid constituents were determined. The determinations were carried out in the cataphoretic cell of J. Northrop, using hydrochloric acid and diluted lactate buffer, respectively. The accuracy of the determination is ± 0.1 pH unit.

Isoelectric point of whole tubercle bacilli (3 strains), pH 3.0.

Isoelectric point of defatted tubercle bacilli, pH 2.8.

Isoelectric point of protein precipitated from watery extract, pH 2.8.

Isoelectric point of lipoid prepared from alcoholic extract, pH 1.5.

Isoelectric point of lipoid prepared from ether extract, pH 1.0.

DISCUSSION

The mechanism of agglutination of whole and defatted (*non-acid-fast*) tubercle bacilli by electrolytes, by normal and by immune serum, resembles closely the agglutination of protein in suspension and the agglutination of gram-negative bacilli. The mechanism of agglutination of lipoid particles prepared from alcoholic extracts of tubercle bacilli differs, inasmuch as salts (sodium chloride, magnesium chloride, calcium chloride) above the concentration 1/100 molecular do not prevent agglutination. Apparently the cohesive force between the lipoidal particles is either not decreased, or not enough decreased to hinder the agglutination.

As the agglutination depends upon the surface of the agglutinated particles, these observations suggest that the surface of tubercle bacilli is of protein nature. This assumption is supported by the fact that the isoelectric points of the whole and the defatted bacteria are approximately identical, while the isoelectric points of the lipoids (alcohol and ether extracts) were found to be different with more than one pH unit.

The acid-fastness does not exclude the possibility of a surface of protein nature. The isoelectric point of collodion particles treated with a protein solution is identical with the isoelectric point of the protein used, yet these collodion particles covered with protein remain acid-fast.

The agglutination of lipoids appears to be specific. The most marked difference between the effect of normal and immune serum can be demonstrated in the agglutination of sensitized lipoid particles by electrolytes, especially by sodium chloride.

SUMMARY

1. The agglutination of two strains of tubercle bacilli and also the agglutination of suspensions of the protein and the lipoid substances of tubercle bacilli were studied.

2. Suspensions of *whole tubercle bacilli* agglutinate by electrolytes when their potential is below −13.0 millivolts. Sodium chloride, calcium chloride and magnesium chloride cause no agglutination, even below −13.0 millivolts as a rule. Some suspensions of strain HD were agglutinated by calcium chloride and magnesium chloride at the concentrations 1/16, 1/32 and 1/64 molecular.

3. The behavior of *defatted* (*non-acid-fast*) tubercle bacilli is similar.

4. Agglutination of *lipoid suspensions* by electrolytes occurs whenever the potential of the particles is reduced below about 15 millivolts, even with sodium chloride, calcium chloride and magnesium chloride.

5. With immune serum, sensitized tubercle bacilli are agglutinated by electrolytes whenever the potential is below 13.0 millivolts, even with sodium chloride, calcium chloride and magnesium chloride.

6. The behavior of the defatted (non-acid-fast) tubercle bacilli sensitized with immune serum is similar.

7. Normal and immune sera decrease the actual potential of lipoid particles; the immune serum is more effective. Both agglutinate the lipoid particles and normal serum decreases the critical potential for agglutination to 7 millivolts. With immune serum, sensitized lipoid particles are agglutinated by sodium chloride at concentrations above 1/1000 molecular, by calcium chloride at concentrations above 1/4000 molecular, and by copper sulphate at concentrations above 1/16000 molecular.

8. The isoelectric points of three strains were found to be between pH 2.8 and pH 3. The isoelectric point of the defatted bacilli is close to pH

2.8. The isoelectric point of lipoid particles prepared from an alcoholic extract is near pH 1.5, that of particles prepared from etheric extract near pH 1. The determinations were made by cataphoresis.

These observations support the assumption of a protein surface of the bacilli.

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COMPLEMENT FIXATION IN TUBERCULOSIS

III. STUDIES ON THE NATURE OF THE ANTIGEN[1]

MAX PINNER

In a previous publication (1) the practical results with Wassermann's antigen for complement fixation in tuberculosis were reported. In the meantime the number of sera tested with this antigen has reached more than 2000; the results remained materially the same. Most of the sera have been tested at the same time with numbers of different antigens. The conclusions drawn in the former paper, based on our experience and on analysis of the literature regarding this question, are fully supported by the larger number of sera and antigens since, and may be restated as follows: The best antigens known so far give only a positive fixation in 60 to 80 per cent of patients with active tuberculosis; the percentage in incipient cases, in which serological help is especially needed, is considerably smaller; and with all tuberculosis antigens positive tests are obtained (in varying percentage) in latent tuberculosis and in patients who have no active tuberculosis and never have had active tuberculous disease. Recently, two excellent and exhaustive reviews (2) have been published concerning the serological diagnosis of active tuberculosis, so that it appears superfluous to go over all the details again. There are, however, three points which have to be emphasized in persuing the literature in question. First, it is a common occurrence that the inventor of an antigen obtains better results with his antigen than do subsequent investigators with the same antigen. Table 1, which could be enlarged at will, gives a few typical examples picked at random.

Differences in technique and in strain of bacillus used may account for this phenomenon, but the author and subsequent investigators have to be taken into consideration in order to arrive at a fair judgment.

Second, publications based on a small number of tests can, for obvious reasons, have only a very questionable statistical value. If, for example,

[1] From the Laboratories of the Municipal Tuberculosis Sanitarium, Chicago, Illinois. Read in part before the Section of Pathology at the annual meeting of the National Tuberculosis Association, Minneapolis, June 17 to 20, 1925.

the specificity of an antigen is claimed because it gave no positive reactions with 8 nontuberculous sera, as has been the case in a recent publication, or its sensitiveness is judged by testing some 70 tuberculous sera, it is not permissible to express the results in terms of percentages.

The third point is the fact that the method of statistical presentation varies very much from one author to another. This makes comparison of results very difficult, and a careful analysis is necessary in order to extract the definite and significant figures. Two examples may illustrate the point. Miller (3) presents the following grouping of his cases: 284 active pulmonary, 113 inactive pulmonary, 228 doubtful cases, 144

TABLE 1

	POSITIVE IN ACTIVE TUBERCULOSIS	POSITIVE IN PATIENTS WITHOUT ACTIVE TUBERCULOSIS
	per cent	*per cent*
1. Miller's antigen:		
Miller (3)	about 97.0	2.9
Stivelman (4)	51.4	32.0
2. Wilson's antigen:		
Wilson (5)	92.0	0
Stoll and Neumann (6)	58.0	3.5
3. Petroff's antigen:		
Petroff (7)	about 94.0	about 7.5
Moursund (8)	15.0	45.0
4. Craig's antigen:		
Craig (9)	96.6	0
McCaskey (10)	77	about 29

nontuberculous sera, 243 positive Wassermann sera. In the third group of 228 patients who are not diagnosed as active tuberculosis, 39 gave a positive fixation; this may, in the worst case, mean either 17 per cent of nonspecific reactions, or 83 per cent of negative fixations in active patients. Obviously, the true figure for positive fixations must be somewhere between Miller's reported 97 per cent and 17 per cent, and the true figure for nonspecific fixations somewhere between 2.9 per cent (Miller's report) and 17 per cent.[2]

[2] Concerning Miller's antigen Zinsser (*Bacteriology*, ed. 5, 1922, p. 609) writes, "It would appear at present that about 70 per cent of the fixation results correspond with clinical findings."

A very similar condition is found in the report by Watkins and Boynton (11) on 6500 cases. They have a group of 521 patients "clinically tuberculous, but not proved," with 64.6 per cent positive fixations; a group of 822 patients "in whom tuberculosis was suspected, but not definitely diagnosed," with 37 per cent positive fixations; and a third group of 554 sera "from patients not examined for tuberculosis," with 33 per cent positive tests. It is evident how the consideration of these three classes of patients will materially alter the percentage figures derived only from the two definitely diagnosed classes of patients as tuberculous with 78 per cent and nontuberculous with 4.2 per cent positive fixations.

The mass of evidence accumulated seems to indicate that no method at the present time gives sufficiently reliable results to warrant the rather time-consuming procedure as a routine test. This is especially true if one considers only those cases in which the clinical symptoms are so slight as to make the diagnosis difficult, and in which the finding of tubercle bacilli does not decide the case. But it appears that some scientific questions were involved in the problem of the antigen which had seldom received very much attention. First of all, it is still an open question which chemical groups of the tubercle bacillus are necessary for an antigen in complement fixation. Although a very great number of different antigens have been in use, very little work has been done to answer this question. Since this study was undertaken in an attempt to contribute to the solution of this problem, and not in order to find a "better" antigen, all the antigens containing whole bacilli or fully undefined mixtures have not been considered. In this category belong the antigens of Miller, of Besredka, and the different ones which have been prepared from tuberculous tissue.

As it appears from the literature at present, the question is whether the antigenic substances ("antigenic" is used only as far as complement fixation is concerned) belong to the water-soluble, the protein, or the lipoid fraction of the tubercle bacillus. Much's opinion is that chemically different fractions of the bacillus react with chemically different antibodies.

LITERATURE

Watery, saline or alkaline extracts have been used for complement fixation by Petroff (7), Corper and Sweany (12), and Seiffert and Meyer (13). Petroff's sodium-hydroxide extract has been abandoned by its author in favor of a glycerin extract. The autolysate of Corper and

Sweany, according to the reports of the authors, gives a slightly lower percentage of positives in active tuberculosis than Petroff's alcohol extract. Seiffert and Meyer, who conducted their experiments on cattle, did not obtain encouraging results with a watery extract with or without the addition of lecithin.

Lipoid-free or protein antigens have frequently been used. It must, however, be emphasized how extremely difficult, if not impossible, it is to obtain, without very severe chemical action, a really lipoid-free tubercle-bacillus fraction. Even the prolonged extraction of bacilli with one or more fat solvents by no means ensures the complete removal of the fatty and lipoidal substances. In this category we can therefore only speak of antigens from which fat and lipoids have been more or less completely removed. Wilson (5) used a saline suspension of tubercle bacilli which were extracted by ether and alcohol; the results with this preparation are recorded in table 1. Wassermann (14) extracted tubercle bacilli for a long time with tetraline, which is supposed to effect "an extraction of lipoids, wax and fat," and he evidently surmises that this extraction is complete with the disappearance of acid-fastness. In the directions which accompany his preparation, manufactured by "Riedel-Berlin," this bacterial powder is designated as "tubercle-bacillus protein." Results obtained with this antigen have been recorded with a review of the literature (1). Blumenthal (15) reports favorable results with a "tubercle-bacillus protein" obtained by extracting bacilli with acetone and ether. Coulthard (16) prepared a protein antigen according to Thompson's antiformin method; it showed practically no antigenic properties. He concludes that "the protein fraction of the tubercle bacillus, then, has only a slight effect, if any, in causing complement fixation with serum antibodies;" he reports, too, that bacilli extracted with acetone and ether were almost inert.

More work than on the first two groups has been done on the group of "lipoidal" antigens, meaning all those preparations, which have been obtained by extracting bacilli with fat solvents, and which contain only or mainly lipoids, fats, and waxes. Kurt Meyer (17) appears to have been the first to study the different lipoidal fractions. He found that the acetone-insoluble fractions of an alcoholic extract are much more effective in complement fixation than any other lipoidal fraction. In this category of lipoidal antigens belong Petroff's (7) methyl-alcohol extract, the antigen of Nègre and Boquet (18) and Craig's (9) preparation, which being an alcohol extract of Besredka's antigen contains not only the

bacillary lipoids but egg lipoids as well. Petroff made a very careful study of the individual lipoids of the tubercle bacillus, such as lecithin, kephalin, sphyngomyelin, cuorin and carnithin; and he arrived at the conclusion that the individual "lipins gave no reactions," but "proteins from tubercle bacilli do not give as strong reactions as an extract which contains proteins and lipins." Lucke (17) was not able to detect any antigenic qualities in tubercle-bacillus wax, but it must be noted that he did not use the alcohol-soluble portion in his preparation. Lewis (20) found "that a common feature of the extractive antigens made from tubercle bacilli is a thermostable, alcohol-soluble constituent." As mentioned above, Coulthard (16) obtained no antigenic preparation in his protein fraction, but an alcohol extract prepared according to the procedure of Boquet and Nègre proved to be strongly antigenic. Wadsworth and Maltaner (21) found too that the final alcohol extraction of a purified antigen was more strongly antigenic and less anticomplementary than the original culture filtrates and watery extracts. In their mode of procedure, however, one would expect that the final alcohol extraction does not only contain the alcohol-soluble parts of the tubercle bacillus but of horse serum as well, since they use horse serum to precipitate the original extractions. It is interesting to note that Seiffert and Meyer and Seiffert (22) obtained their best results with acetone-alcohol extracts from tubercle bacilli, and that they had very similar results with acetone-alcohol extracts prepared from beewax. Further experiences with lipoidal extracts are reported by Citron and Klinkert (23), Thiele and Embleton (24), Warden (25), Dudgeon (26), Osumi (27), and others.

This brief review does not by any means attempt completeness; it is only supposed to show how chemically dissimilar preparations have been in use as antigens, and that it is impossible, from the mere study of the results obtained with these different antigens, to come to a conclusion as to which fraction of the bacillus plays the main part in complement fixation.[3]

[3] The very interesting paper by Wadsworth, *et al.*, (Jour. Immunol., 1925, x, 241) has appeared since the completion of these studies. The authors come to the same conclusions, as here presented, on the chemical nature of the antigen. Their findings, of about 95 per cent of positive fixations in active tuberculosis are remarkably good. But the number of sera examined is too small to form a definite judgment; and, more important, their series of patients comprises only a very few incipient or bacillus-negative cases. The fact that they use as little complement as one hemolyzing unit very likely accounts for the high sensitiveness of the test; yet, at the same time, they obtained about 13 per cent of nonspecific reactions.

METHODS

In order to test a large number of different antigens a standard technique was deemed necessary. Each antigen was first titrated for its anticomplementary and its antigenic value. In the anticomplementary titration saline solution was used instead of negative serum, because there is no way of determining whether a human serum is really entirely free from tuberculosis antibodies. Although this procedure may appear objectionable, it was felt that the saline solution had the advantage over negative serum in being biologically inert and constant; and the nature of this study called only for comparative values. If the anticomplementary value is titrated with negative serum instead of with salt solution one usually finds it to be higher, so as to increase the range. This fact accounts for the smaller ranges that were found in some instances in which previous workers had found a wider range. For the antigenic titrations pooled, 4 plus serum was used. An artificial antituberculosis serum would have afforded a more constant test-object, but the findings of Wassermann (14) may be recalled, which prove that such a serum is serologically not the same as the serum of a tuberculous patient. By pooling three or four sera a higher degree of constancy was obtained. When similar series of antigens had to be tested the same mixture was always used. The technique was as follows: 0.5 cc. of serum diluted 1:10; antigen in amount of 0.5 cc.; complement 1:20, 0.25 cc.; 3/4-hour incubation in water-bath at 37°C.; 0.5 cc. sensitized sheep erythrocytes; 2.5 per cent suspension and two units of hemolysin; second incubation 1/2 hour. Some objections may be raised against this technique, but it was used throughout these studies and offers therefore comparable values. It is the technique employed by Wassermann with his new antigen, which with these experiments were started.

After the titrations the "antigenic quotient" = anticomplementary unit: antigenic unit (as defined by Kolmer) was determined. It was postulated from the beginning that only antigens giving a quotient of 2 or more could be considered for further tests, as it does not seem safe to use an antigen in a higher amount than one-half of the anticomplementary unit. If antigens are used in smaller amounts than the antigenic unit on a larger series of sera, one will occasionally obtain fixations due to the varying strength of antibodies in different sera, but such an antigen will invariably yield a very small percentage of positive fixations with tuberculous sera. The determination of the antigenic quotient as stated

above proved to be a very reliable procedure to determine and compare the antigenic value of the different preparations tested. The sensitiveness of an antigen, as tested on larger series of tuberculous sera, seems to go fairly parallel with this quotient.

The methods used in preparing the different antigens will be explained later in discussing them. Here may only be mentioned that the initial material, unless stated otherwise, was always a mixture of 6 different human and 5 different bovine strains, dried *in vacuo* over phosphorpentoxide, finely pulverized and thoroughly mixed. All extractions with fat solvents have been made in a Soxhlet apparatus. Whenever quantitative comparisons were required, a Soxhlet, containing 50 cc. to the overflow, was used, and the same hot plate. That the antigenic substances of the tubercle bacillus are thermostable has already been demonstrated by Petroff and other workers. It was found that antigens prepared from bacilli dried at 58° or at 80° did not differ in their antigenic strength.

RESULTS

Altogether, more than 70 different preparations have been tested in the above described manner.

AQUEOUS EXTRACTS

The first group of antigens were extracts in water or alkaline solutions: For example, one per cent suspension in 0.2 per cent sodium hydroxide of the mixed and dried bacilli ground in the steel-ball-mill for 8 hours, centrifugated, filtered through a Berkefeld filter and neutralized. Corper's autolysate prepared according to the technique of its authors. A modification of the autolysate in which the bacillary suspension after autolysating for 10 days was ground in the mill for 10 hours and centrifugated. "Residue-antigen" and "nucleoprotein," according to the technique given by Petroff. "Residue-antigen," "nucleo-protein" and autolysate, prepared not from the whole bacilli, but from bacilli which had been subjected to a prolonged extraction with acetone, ether and chloroform. In the same group may be mentioned the "tebeprotein-Toeniessen" (28). This is a preparation obtained by extraction with potassium hydrate after preliminary treatment with a diluted mineral acid. After Berkefeld filtration the alkaline extract is repeatedly precipitated with acetic acid and redissolved. The final alkaline solution

is neutralized. Nitrogen content is 12.3 per cent, biuret and Millon reactions are positive, Molisch's test is negative. It contains no phosphorus and no purin bodies. The last preparation of this group was Much's partial antigen MTbL, which was prepared according to Much's specification; it was tested before and after centrifugation, filtration and neutralization. Not one of the preparations mentioned in this group showed a large enough range between anticomplementary and antigenic unit as to make it fit for an antigen in complement fixation, or, in other words, their antigenic quotients were found to be less than 2.

ANTIGENS AFTER EXTRACTION OF LIPOIDS

Our second group comprises bacilli which have been more or less completely defatted by extracting them for various lengths of time with different fat solvents. A large number of preparations belonging to this group have been made; and, since it would be impossible to mention every single one, the results with this type of antigens will be presented in larger groups. When bacilli were extracted with absolute alcohol (either methyl or ethyl alcohol) the residues invariably showed an antigenic quotient below 2. These extracted powders were always brought into a fine 1 per cent suspension in salt solution, and the whole suspension, as well as the supernatant fluid obtained after centrifugating, was titrated. Acetone extraction leaves a residue, of which the antigenic quotient is usually about 2 or slightly more. As will be mentioned in a later part of this paper, acetone seems to remove some of the anticomplementary substances, as evidenced by the alcoholic extract made after acetone extraction. But a prolonged acetone extraction apparently removes some of the antigenic substances as well. The residue after 20 minutes of acetone extraction has a higher antigenic value than the residue obtained after several hours of extraction.

Extraction with ether or with chloroform at no time leaves a residue with a quotient higher than 2. But at the same time neither of these two fat solvents seems to extract the antigenic material as will be shown later. Dreyer's (29) defatted antigen, for which I am much indebted to Mr. Bullock, was titrated and its quotient found below 2.

Bacilli were subjected to prolonged extraction with alcohol, acetone, ether and chloroform, in an attempt to remove, as completely as possible, all the fatty and lipoidal substances, and such extractions have been extended over weeks; but the final residue always had entirely lost its

antigenic values. Wassermann's tuberculosis antigen, according to its author, belongs in the present group. The bacterial powder suspended in salt solution without the addition of lecithin, gives a quotient of about 2. But if one extracts this powder with absolute alcohol it is found that, in spite of the prolonged extraction with tetraline, it still contains a considerable amount of alcohol-soluble substances. The extracted powder does not retain any antigenic qualities, whereas the alcohol extract has an antigenic quotient of more than 2. The antigenic qualities of Wassermann's antigen are therefore evidently not due to the "tubercle-bacillus-protein," but to the alcohol-soluble fraction; it appears even that the proteins exert a certain depressing or anticomplementary influence. Special attention was given to a preparation very similar in nature to Much's partial antigen MTbA, which was obtained by extracting MTbR with alcohol and ether in a Soxhlet. This antigen gave a very poor titration, but still it was used as an antigen in our routine tests in a dose which was equal to the anticomplementary unit and smaller than the antigenic unit. This was done in order to see whether some of the tuberculous sera might not react only with a protein antigen and not with a lipoidal antigen. But in a series of more than 100 sera none was found which reacted with this protein antigen only. It gave only weak fixations and less than half as many than were obtained with lipoidal antigens.

LIPOIDAL ANTIGENS

Acetone extracts after various times of extraction are not active as antigens. The same is true of ether and chloroform extracts or of saline suspensions of the dried residues of these extracts.

Alcoholic extracts, however, contain the antigenic substances. It was found that extraction with absolute methyl alcohol gives more active substances than extraction with absolute ethyl alcohol. The most uniform results were obtained by extraction with a mixture of equal parts of both alcohols. The antigenic quotient of those extractions is found to be always greater than 2, and frequently as high as 8. It appears, therefore, that by alcohol extraction one obtains more of the antigenic principle than in any other way. And alcohol extraction proved to be a better method of testing the presence of these substances than titration of the unextracted material. It was found in this way that neither acetone, nor ether, nor chloroform extraction, nor their combined action removed the antigenic substances from bacilli, although it reduced the

amount to a certain extent. Acetone extraction removes some of the anticomplementary substances, thus increasing the antigenic quotient of the subsequent alcohol extract, but very long acetone extraction removes a considerable part of antigenic substances, as shown by an alcoholic extract made after 20 hours of acetone extraction which yielded only an antigenic quotient of 2, while after 35 minutes of acetone extraction an alcoholic antigen was obtained with a quotient of 25. But it can be shown that alcohol extraction also removes some of the anticomplementary substances. Fractionated alcohol extraction yields, for example after the first two hours an antigen which is more strongly anticomplementary than the second two-hour fraction; the latter, however, is much less antigenic. It appears, therefore, that some of the anticomplementary substances are very easily extracted by alcohol and acetone as well.

In the next series of experiments the optimal duration for both acetone and alcohol extraction was to be determined. Two grams of dried, mixed and finely powdered bacilli were extracted with 200 cc. of methyl-ethyl alcohol and at short intervals 10 cc. of extract were removed. The volume of the alcohol was kept constant throughout the extraction. All fractions were titrated with the same pooled tuberculous serum. It was found that after one hour the extract already had the highest titre obtainable, although the extraction was chemically far from complete. Ten cubic centimetres of the one-hour extractions left 14.3 mgm. of residue after evaporation, while the 24-hour extraction left 40 mgm. of residue. This does not mean that the substances extracted after the first or second hour are not antigenic, because, if one changes the alcohol after the first two hours, one obtains again an antigenic, though less powerful, extract. This finding suggests the importance of the concentration of lipoidal extracts, which will be discussed later.

In a similar way it was found that an acetone extraction of about 35 minutes, preceding the alcohol extraction, will yield the optimal antigenic value. It was further ascertained that acetone-extracted bacilli need only be extracted with alcohol for 1 to 3 hours to yield the highest antigenic quotient. The most satisfactory method to prepare a lipoidal antigen would therefore be as follows: Two grams of dried bacilli extracted with acetone for about 1/2 to 3/4 of an hour; residue dried and extracted with 200 cc. methyl-ethyl alcohol for about 3 hours. An extract prepared in this manner gave a negative test with sulphosalicylic acid and ninhydrin and contained 10.8 mgm. of nitrogen in 100 cc.

The concentration plays apparently an important part in the efficiency of these lipoidal antigens. An antigen, which had a quotient of 25, was concentrated by evaporation on the waterbath to 1/10 its volume; this concentrated extract yielded a quotient of only 10. Another antigen with the quotient 5 was diluted 1 to 5 with alcohol; the diluted extract had a quotient of less than 1. These findings point to the importance of the fineness of the dispersion. It is possible that the degree of dispersion determines the sensitiveness and specificity of the antigen in a similar way as in the luetic extracts.

These lipoidal antigens appear to be very stable. After standing 2 to 3 months in the icebox, a considerable increase in the antigenic quotient is found with great regularity.

The alcohol-soluble lipoids are not only active antigens in complement fixation, but they are biologically active as well. After 5 intravenous injections of diluted alcoholic extracts, rabbits developed very readily strong complement-fixing antibodies, which reacted with different lipoidal antigens and with Wassermann's tuberculosis antigen. Similar results have been obtained by Dienes and Schoenheit (30). The animals did not, however, react to tuberculin.

MIXTURES OF LIPOIDS AND PROTEINS

A large number of mixtures of lipoids with "lipoid-free" or aqueous extracts were titrated. In no case did the addition of a nonlipoid fraction increase the antigenic value of the lipoidal antigen, but the anticomplementary unit of the mixture was always much smaller. In spite of this finding, such mixtures have been used as antigens in our routine test, because the possibility was perceived that some sera may only react with the mixture of both fractions or with its protein factor. One mixture was tried on a series of 130 patients. It consisted of an alcoholic and an alkaline extract of bacilli. This mixture in no case gave a fixation when the straight alcoholic extract did not react, nor were its fixations stronger. It is not the purpose of this paper to report on the clinical significance of complement fixation in tuberculosis or to discuss the practical value of the lipoidal antigens. Quite summarily it may be stated that the results on many hundreds of patients have been essentially the same with this as with Wassermann's antigen.

DISCUSSION

All the evidence presented in this paper indicates that the alcohol-soluble, acetone-insoluble substances of the tubercle bacillus play by far the most important rôle as antigens in complement fixation. The proteins alone seem to be inert. It must, however, be emphasized that native nondenaturalized proteins, free from lipoids, have never yet been obtained. The proteins, which we are able to isolate from bacilli by fat solvents or by aqueous extraction at room or incubator temperature, have an inhibiting effect on the phenomenon. This is clearly demonstrated in the case of Wassermann's tuberculosis antigen, which (without the prescribed addition of lecithin) has a somewhat smaller range of antigenic activity than the alcohol-soluble lipoids isolated therefrom. Another striking example of the same condition is given by the fact that Much's MTbR, that is, the water-insoluble residue after treatment of the bacilli with lactic acid, gives a quotient of only 2, whereas MTbF, the alcohol extract of the former, has a quotient of 16. That it is possible by simple alcohol extraction to obtain a highly antigenic preparation from a practically inert material, demonstrates that complement fixation cannot prove the presence or absence of an antigenic principle unless it be under very definitely suitable conditions.

It is not permissible to identify or associate "anticomplementary" with acetone-soluble and "antigenic" with alcohol-soluble substances. For, as shown, biological extractions are complete long before chemical extractions have reached an end; secondly, a long acetone extraction removes some of the antigenic principle; and third, if after a short time of alcohol extraction the extract is removed and new alcohol used, the secondary alcohol extraction will show a similar increase in the antigenic quotient as after an acetone extraction. The possibility is perceived that these findings may be suggestive as to the biological structure of the tubercle bacillus.

In comparing the results of these studies with those of previous workers they are found to corroborate the findings of Meyer, Nègre and Boquet, Lewis, Coulthard, Wadsworth, Seiffert and Meyer. Petroff's very interesting studies on the individual lipoids, which proved their antigenic inertia, does not prove that a suitable mixture of all these substances is not antigenic. With his alcoholic antigen he demonstrated that the alcohol-soluble substances are highly active. Lucke did not use the alcohol-soluble substances in his work; his negative results, therefore, corroborate in an indirect way the findings presented here.

Corper's autolysate and Petroff's sodium-hydroxide extract undoubtedly contain some of the lipoidal substances in fine dispersion. For the fact that they showed a much poorer antigenic value than purer lipoidal extracts a possible explanation has been advanced above.

Wilson's results could not be duplicated in these studies. Considering how very difficult it is to extract all lipoidal substances quite completely from tubercle bacilli, it may be that his vaccine still contained some of the alcohol-soluble fraction. Wassermann's antigen has been shown to owe its antigenic strength exclusively to its alcohol-soluble fraction, and belongs therefore to the group of lipoidal antigens.

SUMMARY

1. Comparative titration of more than 70 preparations derived from tubercle bacilli proved that the main complement-fixing substances are closely associated with that fraction of the bacilli which is practically insoluble in acetone, ether and chloroform and soluble in alcohol.

2. It is felt that no proof has been given that the alcohol-soluble substances contain the only antigenic principle, but no tubercle-bacillus extract from which these substances have been removed retained a sufficient amount of antigenic power to make it fit as an antigen in complement fixation.

3. Evidence has been introduced that the degree of dispersoin plays an important rôle in the antigenic value of lipoidal tuberculosis antigens.

4. The protein substances exert an inhibiting influence on the lipoids in complement fixation.

5. No evidence has been found for the assumption that certain sera from tuberculosis patients react only with protein and others only with lipoidal antigens.

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THE EFFECT OF MERCUROCHROME IN EXPERIMENTAL TUBERCULOSIS OF THE RABBIT[1]

H. J. CORPER, SAUL MEBEL AND ROSE SILVER

Mercurochrome-220 soluble, dibrom-oxymercuryfluorescin or its sodium salt, the latter containing 26 per cent of mercury, was introduced as an antiseptic by Young, White and Swartz (1) in 1919 for use in the genitourinary tract. The solution is stable, and not affected by moderate heat or exposure to air. Urine of pH 6.4 or less gives no precipitate with mercurochrome and a 1 per cent solution mixed with an equal volume of a medium rich in protein, such as hydrocele fluid, is free from precipitation. The advantages noted for the drug are its ready penetration of the tissues, lack of irritation, high germicidal activity, ready solubility in water and stability of its solution, and sufficiently low toxicity to avoid systemic effects from the small amount of the drug that may be absorbed. A solution of 1 to 1000 kills B. coli and Staphylococcus aureus in urine in one minute, while 1 per cent is tolerated by the human bladder for from one to 3 hours without irritation, and 1 per cent solutions show no irritating qualities when used in the conjunctival sac of rabbits. Clapp and Martin (2) used a 2 per cent solution in the human conjunctiva with impunity, and with only a slight burning effect for the first few seconds, which was verified by Lancaster, Burnett and Gaus (3), who instilled mercurochrome repeatedly in 1 or 2 per cent solution and in gonorrheal ophthalmia used as strong as 10 and 5 per cent solutions. Bray (4) reported favorable results with 1 per cent mercurochrome in erosion of the *os uteri*, abscess of the vaginal floor and acute cystitis, and 0.2 to 0.4 per cent solution in acute empyema, and concludes that the low toxicity of mercurochrome-220 renders it a valuable antiseptic aid in a variety of infections, including those in cavities where drainage is not free. Likewise, Ockerblad (5) reported good results from the injection of 2 per cent solutions into the seminal vesicles.

In 1921 Young, White and Swartz (6) reported further favorable

[1] From the Research Department, National Jewish Hospital for Consumptives, Denver, Colorado.

results on the use of mercurochrome in urethritis, cystitis, pyelitis, chronic discharging sinuses, etc., but stated that "we do not know why a 0.5 per cent solution should prove irritating in an occasional case (of cystitis) and not at all irritating in almost all other cases." "A 1 per cent solution may be injected gently into a fistula with impunity, daily." They recorded reports from sanatoria for tuberculosis attesting its value in various forms of discharging sinuses and fistulas.

Johnson (7) reported 5 cases of perforative appendicitis in which he introduced 10 cc. of 2 per cent solution into the region of the abscess without evidence of peritoneal irritation or sepsis. In 1922 Piper (8) injected mercurochrome in 1 per cent solution intravenously into rabbits in amounts of 5 to 7 mgm. per kilo on three successive days, then with a day's interval and repeated for 14 days, without affecting the red or white blood cells. A rabbit infected with streptococci and treated with mercurochrome was benefited, while the control succumbed to the infection. Encouraging results were obtained from its intravenous use in certain patients. Its greatest value is believed to lie in its use in local infections.

In more elaborate animal tests Hill and Colston (9) found that after a single intravenous injection of rabbits with 1 to 10 mgm. of mercurochrome per kilo the urine becomes bacteriostatic, and in some cases bactericidal for B. Coli, the action being just as marked following small as large doses, due to its elimination by way of the kidney after small doses and its intestinal elimination after larger doses.

The results of Piper and of Hill and Colston encouraged Young and Hill to use mercurochrome intravenously with success in man for the treatment of septicemia and local infections (10), and later Young and Birkhaug (11) used it in two cases of severe scarlet fever complicated with erysipelas and streptococcus septicemia. Hill and Bidgood (12) studied the effect of the intravenous injection of mercurochrome on the kidney of the rabbit, and found that there is no actual destruction of tubular epithelium after doses as high as 7.5 mgm. per kilo body weight, but with 10 mgm. per kilo there is a definite renal damage. Repeated injections do not cause any added damage, and can be given as often as twice a week with safety, provided a dose of 5 mgm. per kilo is not exceeded. The slight damage is not irreparable, and at the end of two months the kidneys show no evidence of any previous lesion.

From the available evidence in the literature it would seem that mercurochrome-220 soluble is innocuous locally in concentrations as

high as 2 per cent, except for a dissenting report by Rolnick (13) in the latter part of 1924, which appeared when our own studies were nearing completion. Vasotomies were performed on 20 dogs, and the vasa of 10 were injected with 2 per cent and of the other 10 with 3 per cent mercurochrome. Of the 10 vasotomies with the 2 per cent solution, one was patent, and in 3 others occlusion was due to filling up of the lumen with mercurochrome and detritus. The other 6 were occluded as a result of scar-tissue formation, so that only 1 of 7 was patent. Of those with 3 per cent mercurochrome, 1 was patent, 3 occluded with mercurochrome and debris, and the other 6 occluded by scar-tissue, so that here also only 1 of 7 was patent.

Recently Hirschfelder, Malmgren and Creavy (14) by means of an ingenious method, demonstrated that mercurochrome penetrated gradually from the blood into the edema fluid in paraphenyldiamin edema, but when intravenous doses proportional to those used clinically are employed it does not render edema fluid bactericidal or bacteriostatic for B. Coli or staphylococcus.

In tuberculosis DeWitt (15) has found that mercurochrome-220 inhibits the growth of tubercle bacilli on glycerol-agar completely, in a dilution of 1 to 5000, and partially at 1 to 10,000. In a dilution of 1 to 100, but not in higher dilutions, it killed tubercle bacilli in 24 hours, so that they would not infect guinea pigs. In guinea pigs inoculated with tuberculosis (16) treatment with mercurochrome seemed to delay considerably the development of the disease; but marked generalized tuberculosis occurred in all the animals that lived long enough. Upon subcutaneous injection mercurochrome (concentration not given) caused infiltration, induration, necrosis and ulceration of the tissues around the point of injection. Nontuberculous animals treated in the same way showed no toxic effects, except the local features described above.

Numerous personal requests for information on the possibility of using mercurochrome in tuberculous empyemas and in tuberculous pulmonary cavities, as well as by intravenous injection for tuberculosis, prompted the following study. The seemingly innocuous nature of mercurochrome directly applied to the tissues, as is evident from the literature which appeared before this study was initiated and before Rolnick's contribution, made it seem desirable to obtain further evidence of its value in tuberculosis by local or intravenous administration.

THE LOCAL EFFECT OF MERCUROCHROME

Since the concentration of mercurochrome which can be used locally with success in the treatment of tuberculosis must depend on its tissue destructive action, on the one hand, and its bacteriostatic or bactericidal action on the other, and no accurate information on the former was available, the local tissue toxicity of mercurochrome was determined in normal dogs by intracutaneous, intratracheal and intrapleural injection. For the purpose of determining the effect of the intracutaneous injections of different concentrations of mercurochrome, four dogs were given 0.2 cc. of 2, 1 and 0.5 per cent mercurochrome, respectively, in duplicate in the skin on both sides of the shaved abdomen, each dog receiving 6 injections; and 4 other dogs were likewise given intracutaneous injections of 0.2 cc. of 0.1, 0.05 and 0.01 per cent mercurochrome. Since the changes resulting from the intracutaneous injection of the mercurochrome were almost identical, except for slight individual variations occurring in the 4 duplicate dogs, the findings in only one of the dogs will be described.

The site of injection of 0.2 cc. of 2 per cent mercurochrome assumed the red-orange hue of the mercurochrome immediately after injection, with no other appreciable reaction visible to the naked eye until 24 hours later, when there had developed a purple, raised ring of congestion (about 0.5 cm. wide) around the deeply tinted mercurochrome (1 to 2 cm.) central site of injection. There did not appear to be any diffusion of the mercurochrome from the intracutaneous site of injection beyond that noted immediately after injection, nor was there any fading of the mercurochrome tint in the tissues at this time. This persisted for 3 days after injection, when the ring of congestion around the mercurochrome faded and there remained the deeply stained centre with an elevated, indurated (0.5 cm.) margin, which persisted until the fifth to seventh day, when the mercurochrome-tinted epidermis sloughed out and left an ulcer with undermined edges and a pale pink necrotic floor. Gradually, the pink debris at the bottom of the ulcer was replaced in about 12 days by an unstained young granulation-tissue floor, which healed in from three to five weeks.

The site of injection of the 0.2 cc. of 1 per cent mercurochrome differed from the lesion produced by the 2 per cent solution only in size, and slightly in the rate of healing of the resultant ulcer. The site of injection of the 0.2 cc. of 0.5 per cent solution of mercurochrome also assumed the orange hue of mercurochrome immediately after injection,

which persisted until ulceration took place after about 7 days. After 24 hours there was a distinct ring of edema around the entire orange-colored site of injection, which persisted until ulceration, which resulted from the sloughing out of the entire mercurochrome-stained area, leaving a small (slightly less than 1 cm. in diameter) ulcer with undermined edges and a faint pink floor of tissue debris which later discharged. Healing occurred in about 3 to 4 weeks by granulation-tissue repair.

The site of the intracutaneous injection of 0.2 cc. of 0.1 per cent mercurochrome presented an immediate pink, elevated area, about 4 mm. in diameter, on the first day, which persisted for about 3 days, after which there remained only a small, elevated, indurated nodule free from mercurochrome color. All perceptible evidences of the injection were absent about 1 week after injection. The 0.05 per cent solution of mercurochrome produced a lesion resembling that with the 0.1 per cent, but a little smaller (2 to 3 mm. in diameter) and persisting for a shorter time (3 to 4 days). The injection of 0.2 cc. of 0.01 per cent mercurochrome produced no appreciable macroscopic changes perceptible after 24 hours, nor did it tint the tissues at the site of injection.

Microscopic examination of sections obtained from the areas of the skin ulcers, 12 and 22 days after the intracutaneous injection of 0.5 and 1.0 per cent mercurochrome solutions, revealed a skin ulcer, with a granulation-tissue floor in various stages of scar-tissue formation and undermined epithelial edges.

It is evident from the above experiment that the intracutaneous injection of 0.2 cc. of concentrations as low as 0.5 per cent of mercurochrome-220 soluble result in marked tissue destruction, and 0.1 or 0.05 per cent solutions produce evident though only transient pathological tissue changes.

Since a suitable antiseptic solution, to be used in the local treatment of pulmonary tuberculosis or to be locally applied in tuberculous cavities, should be practically innocuous to the normal pulmonary parenchyma, because of the dangers of aspiration of the solution into normal lung tissue (17), it seemed advisable to test the effect of various concentrations of mercurochrome on the lung after intratracheal injection. For this purpose, a series of 21 dogs were intratracheally injected. Five received 2.5 or 5 cc. of 2 per cent mercurochrome;[2] five, 2.5 or 5 cc. of

[2] All solutions were freshly prepared, before use, from crystalline mercurochrome-220, obtained from Hynson, Westcott and Dunning of Baltimore. A 2 per cent solution was prepared with distilled water and brought to a gentle boil, and the desired dilutions were derived from this solution with sterile distilled water or sterile 0.9 per cent sodium-chloride solution without further boiling.

1 per cent mercurochrome; three, 5 cc. of 0.5 per cent; three, 5 cc. of 0.1 per cent; three, 5 cc. of 0.01 per cent; and two, as controls, 10 cc. of sterile physiological salt solution.

Two dogs received an intratracheal injection of 5 cc. of 2 per cent mercurochrome, and died after 20 hours and 3 days, respectively, from acute hemorrhagic pneumonia, involving in the former the lower and middle lobes of the right lung, and in the latter the entire lower lobe and part of the upper lobe of the left lung besides a few discrete areas in the middle and lower lobes of the right lung. Three dogs received an intratracheal injection of 2.5 cc. of 2 per cent mercurochrome; one was examined after 1 hour, one died 22 hours after injection, and one lived 12 days and was then examined. In the first dog, examined after 1 hour, there was early pneumonic consolidation of both lower lobes. The second dog, dying 22 hours after injection, had a marked, acute, hemorrhagic pneumonia of both lower lobes; and the third dog, examined after 12 days, revealed an area of consolidation in the upper portion of the lower lobe and the lower portion of the upper lobe of the left lung, and in the posterior portion of the upper lobe of the right lung.

Two dogs received an intratracheal injection of 5 cc. of 1 per cent mercurochrome, and 1 was examined 24 hours after injection and revealed a complete hemorrhagic pneumonia of the lower portion of the lower lobe of the left lung, with a few pneumonic areas in the upper lobe of the right lung. The second dog died 22 days after injection, and showed an organizing pneumonia, with multiple focal abscesses in the lower lobes of both lungs and a few areas of consolidation scattered throughout the upper lobes. Three dogs received an intratracheal injection of 2.5 cc. of 1 per cent mercurochrome. One of these dogs was examined 1 hour after injection, when there was found an early acute pneumonia of the lower lobes of both lungs and of the middle lobe of the right lung. Another dog was examined 24 hours after injection, and was found to have acute hemorrhagic pneumonia of the upper lobe of the right lung and of both lower lobes. The third dog was examined six days after injection, and showed a resolving and organizing pneumonia of the lower lobe of the left lung.

All of the lungs of the above dogs were sectioned and examined microscopically. Briefly stated, the immediate microscopic effects of mercurochrome in 2 and 1 per cent solution were a pronounced intraälveolar hemorrhage, with marked desquamation of the alveolar epithelium.

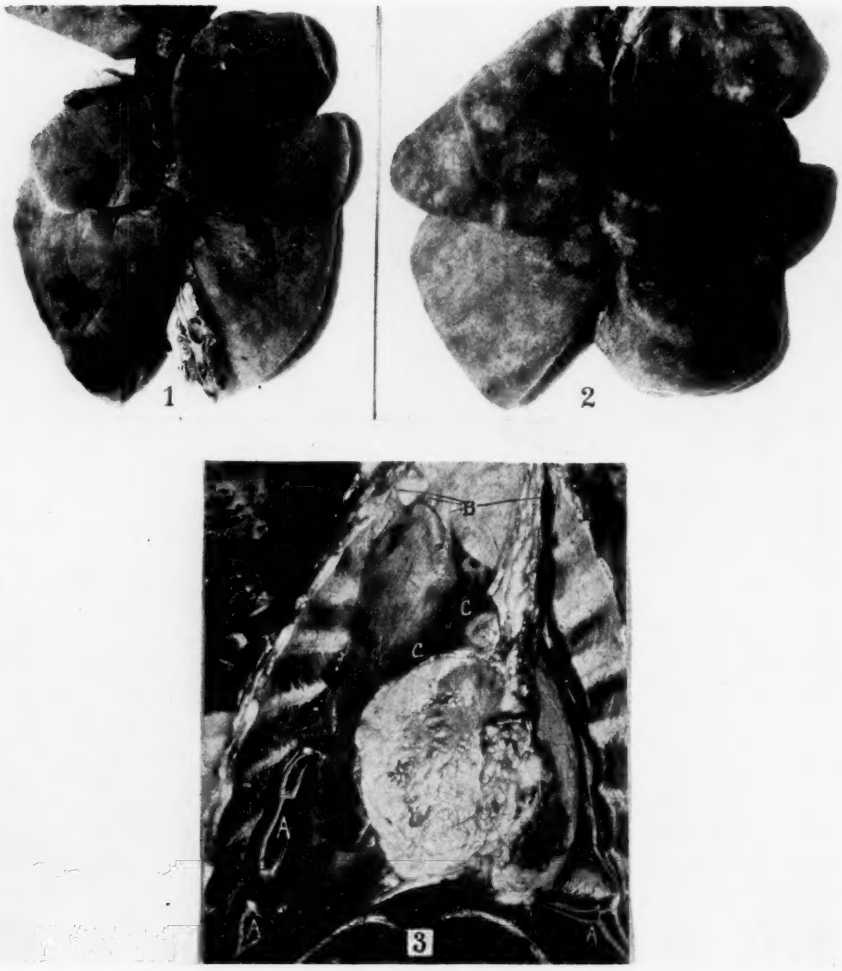

FIG. 1. LUNGS OF DOG 24 HOURS AFTER INTRATRACHEAL INJECTION OF 5 CC. OF 1 PER CENT MERCUROCHROME SOLUTION, SHOWING ACUTE PNEUMONIC AREAS OF MERCUROCHROME LOCALIZATION (DARK AREAS)

FIG. 2. LUNGS OF DOGS 22 DAYS AFTER INTRATRACHEAL INJECTION OF 5 CC. OF 1 PER CENT MERCUROCHROME SOLUTION, SHOWING AREAS OF CONSOLIDATION AND ABSCESS FORMATION (LIGHT AREAS)

FIG. 3. CHEST OF DOG 24 HOURS AFTER RIGHT-SIDED INTRAPLEURAL INJECTION OF 10 CC. OF 0.5 PER CENT MERCUROCHROME SOLUTION

Note the hemorrhagic pleural effusion, *A*, numerous fibrin strands, *B*, and pneumonic involvement of anterior pendant portions of the lung, *C*

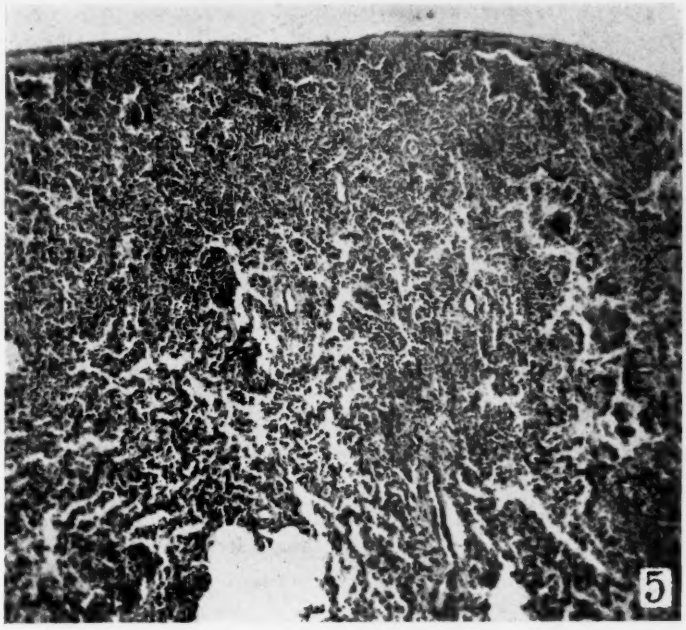

FIG. 4. LUNG OF DOG 3 DAYS AFTER INTRATRACHEAL INJECTION OF 5 CC. OF 2 PER CENT MERCUROCHROME SOLUTION

Note profound hemorrhage and destruction of pulmonary parenchyma

FIG. 5. LUNG OF DOG 3 DAYS AFTER INTRAPLEURAL INJECTION OF 10 CC. OF 0.5 PER CENT MERCUROCHROME SOLUTION

Note pneumonic infiltration below pleura with marked intraälveolar hemorrhage

Early, the alveoli remain intact, and bordering the hemorrhagic areas there were found alveoli filled with serum and fibrin and a few cellular elements, including leucocytes, and desquamated alveolar epithelium. After 24 hours the hemorrhagic areas became more pronounced, and in many places all alveolar structure was absent, and the conspicuous cell was the erythrocyte, with a few polymorphonuclear leucocytes interspersed. The smaller bronchioles did not seem to be appreciably affected, unless they were found in the areas of most pronounced hemorrhage where they become practically obliterated. After 6 days the nucleated cells increased in number, and numerous endothelial leucocytes were seen. There were also small focal abscesses in the more affected regions. The erythrocytes seemed especially well preserved. Later the affected areas gradually took on the appearance of a granulation tissue, with numerous endothelial and connective-tissue cells among which a few erythrocytes were still interspersed. The smaller bronchioles were, in many cases now filled with a cellular debris, consisting mainly of polymorphonuclear leucocytes and a few endothelial cells. The lining of the bronchioles appeared intact and little affected. The picture was, to a great extent, that of a slowly resolving pneumonia, with delayed resolution and granulation-tissue formation in places. Focal abscesses seemed to be conspicuous in certain sections examined at the later intervals.

Three dogs received by intratracheal injection 5 cc. of 0.5 per cent mercurochrome solution. The lungs of one dog were examined 24 hours after injection, and there was found a complete hemorrhagic consolidation of the posterior portion of the lower lobe of the right lung, and a small similar area posteriorly, in the lower lobe of the left lung. The lungs of the second dog were examined 3 days after injection, and there was found an acute hemorrhagic pneumonia of the anterior portion of the upper lobe of the right lung, the posterior portion of the lower lobe and the anterior lower portion of the upper lobe of the left lung. In the lungs of the third dog, examined 2 weeks after injection, there were areas of hemorrhagic consolidation in the accessory lobe and in the middle lobe of the right lung.

Microscopic sections of the lungs of the three dogs given 0.5 per cent mercurochrome strikingly resembled those given 1 and 2 per cent mercurochrome, there being an early, marked intraälveolar hemorrhage, with obliteration of alveolar walls and subsequent granulation-tissue healing, and the formation of small focal abscesses. Three dogs re-

ceived an intratracheal injection of 5 cc. of 0.1 per cent mercurochrome solution, and the lungs were examined after 24 hours, 4 days and 1 week, respectively. In 24 hours there was a small area of consolidation in the lower lobe of the left lung, and another in the lower portion of the upper lobe of the left lung, while after 4 days there were numerous small areas of acute pneumonia scattered throughout both lungs. In the dog examined after 1 week there was a large pneumonic area in the upper portion of the lower lobe of the left lung and a few smaller areas in the upper lobe. Microscopically, there was less hemorrhagic infiltration, and more desquamative and proliferative cellular reaction in the alveoli in these lungs than in those after the higher concentrations of mercurochrome. As a whole there was more of a proliferative than a destructive tissue tendency in animals given this concentration, with more rapid resolution and repair, although the tissue injury produced by the mercurochrome was still distinct.

An intratracheal injection of 5 cc. of 0.01 per cent mercurochrome was given to three dogs that were examined after 24 hours, 3 days and 1 week. Aside from a few suspicious areas of congestion found macroscopically in the 24-hour and 3-day lungs, nothing else was found. The 1-week lungs were entirely negative. Microscopically, a few areas were found in which the alveoli contained a large amount of fibrin, a few leucocytes, endothelial cells and desquamated alveolar epithelial cells.

The lungs of the 2 dogs given, as controls, an intratracheal injection of 10 cc. of sterile 0.9 per cent sodium-chloride solution were negative, both macroscopically and microscopically, after 1 and 3 days.

In order to determine the possibility of using mercurochrome as an antiseptic in tuberculous empyema, the effect of mercurochrome on the normal pleura was studied in 8 dogs given intrapleural injections of 0.1, 0.5, 1 and 2 per cent solutions.

Two dogs were given an intrapleural injection of 5 cc. of 2 per cent mercurochrome solution on the right side, and upon examination, 24 hours after injection, one of the dogs had a marked hemorrhagic pleurisy with numerous fibrin deposits on the pleura of both sides and about 25 cc. of bloody fluid in the right and left pleural spaces. The lungs, especially in the anterior pendant portions on both sides, were consolidated, presenting acute hemorrhagic pneumonia. The second dog was examined on the third day after injection, and there was found a large gelatinous, mercurochrome-stained, hemorrhagic area in the thoracic fascia and muscles at the site of injection. There was also a small

amount of hemorrhagic fluid in both pleural cavities, with deposits of fibrin on the pleural surfaces, and numerous superficial pneumonic and inflammatory areas. Evidently, the greater part of the solution did not enter the pleural cavity but was injected into the chest wall.

Two dogs were given a right-sided intrapleural injection of 5 cc. of 1 per cent mercurochrome solution, and were examined one and six days after injection. The dog examined after one day presented a marked pleurisy with hemorrhagic effusion and fibrinous pleuritis. The right pleural cavity contained 22 cc. and the left 19 cc. of bloody fluid. On all the pleural surfaces of both sides of the chest were heavy deposits of fibrin. The lungs were congested and pneumonic in places. The second dog, examined 6 days after injection, revealed no pleural fluid but showed numerous fibrin strands between the parietal and visceral pleurae and to the diaphragm, as well as between the lobes of the lungs on both sides. The pleural surfaces were dull and congested, and the lungs presented a number of pneumonic areas in the stage of resolution.

Two dogs were given right-sided intrapleural injections of 10 cc. of 0.5 per cent mercurochrome, and their chests were examined one and three days after injection. The dog examined one day after injection presented a marked hemorrhagic pleurisy, with 34 cc. of bloody fluid on the right side and 20 cc. of a similar fluid on the left. There were numerous fibrinous strands on the pleurae on both sides, and the anterior pendant portion of the lungs presented a distinct hemorrhagic pneumonia similar to that noted after the intratracheal injection of mercurochrome. The second dog, examined 3 days after injection, also presented similar findings, with 32 cc. of hemorrhagic fluid in the right pleural space and 30 cc. in the left. There were numerous heavy fibrinous strands around the apices, and from the lungs to the diaphragm, and the anterior lung borders were pneumonic as in the above animals.

The two dogs given right-sided intrapleural injections of 10 cc. of 0.1 per cent mercurochrome were examined one and two days after injection, and had, in both cases, a dry fibrinous pleurisy bilaterally. The pleura was dull, and speckled with fibrin masses, the condition being slightly more marked on the right than on the left. No appreciable fluid was found in the pleural space of either dog.

On microscopic examination the lungs of the above dogs, receiving 2, 1 and 0.5 per cent mercurochrome solution injected into the pleural

cavity on the right side, presented an acute hemorrhagic pneumonia, resembling that noted following the intratracheal injection of the same concentrations of mercurochrome, although the pulmonary changes were less marked for the same concentration following intrapleural than following intratracheal injection. In view of the fact that in the dog there is ready access of fluid from one side of the chest to the other following intrapleural injection, the pulmonary effects were not limited to the lung of the side of injection but were also found in the opposite lung, though in many cases slightly less severe. It is plain from the foregoing experiments, and the fact that DeWitt found mercurochrome inhibitory to the tubercle bacillus *in vitro* only in dilutions above 0.02 per cent and that it required exposure to 1 per cent for 24 hours to kill them, that little could be expected from mercurochrome-220 soluble as a local antiseptic in tuberculosis if it were to be used directly, either in pulmonary cavities or by intrapleural injection for empyemas or probably sinuses.

The apparently innocuous nature of mercurochrome upon intravenous injection of appreciable amounts, as attested by the literature, and the possibility of other factors entering into consideration in this mode of treatment, made it seem advisable to verify and elaborate on the findings obtained with guinea pigs following subcutaneous treatment, by using rabbits treated by the intravenous injection of mercurochrome.

The rabbit was chosen as an experimental animal, to determine the effect of the intravenous injection of mercurochrome on tuberculosis, for a number of reasons. The toxicity of mercurochrome has been elaboratorely studied, and it has been found that 5 mgm. per kilo body weight intravenously can be administered repeatedly to this animal without appreciable harm; intravenous injections into the ear veins can be repeatedly and easily given; and the tuberculosis resulting from the intravenous injection of either human or bovine bacilli is predominantly pulmonary.

A total of 54 rabbits were used, half of them being infected by the intravenous injection of a suspension of a virulent bovine tubercle bacillus (*Bov. Vir.*) and the other half receiving an intravenous injection of a suspension of virulent human tubercle bacillus (*Gluckson*). The human bacilli were given in amounts of 10, 1 and 0.1 mgm. per pound body weight, and the bovine bacilli in amounts of 0.01, 0.000,1 and 0.000,001 mgm. per pound body weight. The experiment was further

TABLE 1

The effect of intravenous mercurochrome-220 soluble treatment, initiated immediately after infection, on tuberculosis in the rabbit

TREATMENT GIVEN RABBITS	ORGANS RECORDED	5 MGM. PER KILO GIVEN INTRAVENOUSLY DAILY FOR 2 WEEKS. TREATMENT INITIATED 1 HOUR AFTER INFECTION					
		Bovine virulent			Gluckson		
		0.01*	0.000,1	0.000,001	10	1.0	0.1
Mercurochrome I	Lung	42†	44	25	3P	4P	0
	Liver	0	0	0	0	0	0
	Spleen	41	—	11	0	0	0
	Kidney	11	11	11	0	0	0
	Interval after infection	K‡ 22d	K 41d	K 57d	K 22d	D 20d	K 60d
Mercurochrome II	Lung	42	48	19	4P	0	0
	Liver	0	0	0	0	0	0
	Spleen	41	12	0	0	0	0
	Kidney	11	12	0	0	0	0
	Interval after infection	K 22d	D 41d	K 57d	K 22d	D 25d	D 42d
Controls untreated††	Lung	41	33	5§	5**	32	0
	Liver	—	0	0	0	0	0
	Spleen	—	11	11?	0	0	0
	Kidney	—	11	11?	0	?	0
	Interval after infection	D 21d	D 32d	D 57d	D 3d	K 54d	K 6m

* The figure designates the amount in milligrams per pound body weight injected intravenously for infecting.

† The tuberculous involvement of an organ is designated according to the number of tubercles present by the left-hand digit and according to the size of the tubercles by the right-hand digit, according to the following gradations:

The left-hand digit grades are: 0 indicates that no tubercles were found macroscopically; 1 indicates that the tubercles were rare, a few (1 to 20) in the entire organ; 2 indicates tubercles were moderate in number, from 20 to 100 in the entire organ; 3 indicates tubercles were relatively numerous (5 or more to the square centimetre); 4 indicates that tubercles were very numerous (over 20 to the square centimetre); 5 without a modifying digit indicates a massive diffuse tuberculous involvement of the entire organ.

The right-hand digit grades are: *P* indicates that the tubercles are smaller in diameter than 1 millimetre; sizes exceeding 1 millimetre are designated by the respective number up to 9, indicating the diameter of the average tubercles found in the organ.

‡ *K* indicates that the animal was killed for examination on the day *d* or month *m* designated after infection and *D* that the animal died.

§ In this animal it was questionable whether all the lesions found in the lungs and other organs were due to tuberculosis or to some other disease in the rabbit.

** In this animal there was a caseous pneumonia of the upper lobes of both lungs.

†† The studies recorded in tables 1, 2 and 3 were performed at the same time so that the controls in these three tables apply to all.

subdivided so that one set of each receiving the same infecting dose was treated with mercurochrome (5 mgm. per kilo) one hour after infection and daily for 2 weeks thereafter, the object being to have the mercurochrome act before the bacilli lodged in the tissues and became intracellular; another set was treated beginning one week after infection and

TABLE 2

The effect of intravenous mercurochrome-220 soluble treatment, initiated one week after infection, on tuberculosis in the rabbit

TREATMENT GIVEN RABBITS	ORGANS RECORDED	5 MGM. PER KILO GIVEN INTRAVENOUSLY DAILY FOR 2 WEEKS. TREATMENT INITIATED 1 WEEK AFTER INFECTION					
		Bovine virulent			Gluckson		
		0.01	0.000,1	0.000,001	10	1.0	0.1
Mercurochrome I	Lung	41	21	28	4P	11	12
	Liver	0	0	0	0	0	0
	Spleen	31	0	4P?	0	0	—
	Kidney	11	0	0	0	0	—
	Interval after infection	K 21d	D 26d	K 57d	D 21d	D 51d	K 6m
Mercurochrome II	Lung	41	35	16	4P	5	0
	Liver	0	0	0	0	0	0
	Spleen	—	11	11	0	0	0
	Kidney	—	23	0	0	0	0
	Interval after infection	D 19d	K 41d	K 57d	D 22d	D 20d	K 6m
Controls untreated	Lung	41	35	23	32	41	?
	Liver	0	0	0	0	0	0
	Spleen	21	11	0	0	0	0
	Kidney	11	0	0	0	?	0
	Interval after infection	D 21d	D 41d	K 56d	K 22d	K 54d	K 6m

daily thereafter for two weeks, when the bacilli had lodged in the tissues and were intracellular; and in the third set 2 mgm. per kilo of mercurochrome was used daily throughout the entire experiment up to eight weeks, beginning one day after infection. The results of the treatment of the various sets with mercurochrome are recorded in tables 1, 2 and 3.

No appreciable effect from the intravenous injection of mercurochrome

upon tuberculosis in rabbits resulting from the intravenous injection of a suspension of virulent human or bovine tubercle bacilli was noted, regardless of whether the mercurochrome injections were begun immediately or one week after infection. It is interesting in this respect

TABLE 3

The effect of intravenous mercurochrome-220 soluble treatment, in small doses, initiated one day after infection, on tuberculosis in the rabbit

TREATMENT GIVEN RABBITS	ORGANS RECORDED	2 MGM. PER KILO GIVEN INTRAVENOUSLY DAILY FOR 8 WEEKS. TREATMENT INITIATED 1 DAY AFTER INFECTION					
		Bovine virulent			Gluckson		
		0.01	0.000,1	0.000,001	10	1.0	0.1
Mercurochrome I	Lung	42	5	35	4P	31	0
	Liver	0	0	0	0	0	0
	Spleen	31	11	31	?	0	0
	Kidney	21	0	12	0	11	0
	Interval after infection	K	K	K	D	K	D
		22d	41d	57d	20d	54d	12d
Mercurochrome II	Lung	42	—	15	4P	0	0
	Liver	0	0	0	0	0	0
	Spleen	—	—	21	0	0	0
	Kidney	21	—	0	0	0	0
	Interval after infection	K		K	D	D	D
		22d	—	57d	30d	12d	50d
Controls untreated	Lung	41	34	—	4P	0	11
	Liver	—	0	—	0	0	0
	Spleen	?	12	—	0	0	0
	Kidney	—	0	—	0	0	0
	Interval after infection	D	D	—	K	D	K
		20d	48d		21d	21d	6m

to note the recent findings of Hirschfelder, Malmgren and Creavy (14) in that, after giving an intravenous injection of 5 mgm. mercurochrome per kilo to paraphenylendiamine intoxicated rabbits, concentrations of 1:5000 to 8000 are present in the edema fluid after 45 minutes (equal to that in the plasma).

SUMMARY AND CONCLUSIONS

Mercurochrome-220 soluble given intravenously daily over long periods has no appreciable effect on the tuberculosis developing in rabbits after the intravenous injection of suspensions of virulent human or bovine tubercle bacilli, regardless of whether treatment is begun immediately or a week after infection. The local tissue toxicity of mercurochrome-220 soluble in relatively low concentrations and its poor tuberculocidal and bacteriostatic efficiency would not seem to justify its use in the local treatment of tuberculous empyemas or pulmonary cavities.

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